

**Reproductive styles of Osteoglossomorpha with emphasis on
Notopterus notopterus and *Osteoglossum bicirrhosum***



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Dedicated to

Prof. Dr. Frank Kirschbaum
&
Dr. Peter Bartsch

Thousand words are never enough to express my gratitude.
It's been a long journey, a precious and unforgettable experience in my life.

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SUMMARY

The Osteoglossomorpha represent a basal group of teleostean fish comprising taxa with a mixture of both plesiomorphic and apomorphic characters of reproduction and ontogenetic development. Concerning reproductive styles and ontogenetic development of this group, there are still very limited data available so far. Some information on different aspects of reproduction does exist. Most in depth studies are available for mormyrids, but detailed descriptions and experimental data remain scarce in the other groups as in *Notopterus notopterus* and *Osteoglossum bicirrhosum*.

This study will describe in detail for the first time the ontogenetic development of these two species in laboratory-reared specimens. Breeding experiments aimed at potential environmental triggers for gonad development or courtship behaviour of five species of three families of the order Osteoglossiformes: Mormyridae (*Petrocephalus soudanensis* and *Petrocephalus catostoma*), Pantodontidae (*Pantodon buchholzi*), and Notopteridae (*N. notopterus* and *Xenomystus nigri*). For study of Osteoglossidae (*O. bicirrhosum*) a five-month field work took place in Colombia.

Only *N. notopterus* succeeded in the breeding experiment. Experimental data demonstrated that the environmental factors decreasing conductivity, slight variation of temperature, and water level have no influence on gonad development or courtship behaviour in *N. notopterus*. Spawning occurs during day time at a temperature of 25–28 °C. Newly spawned 3.8–4 mm adhesive eggs are guarded by the male until hatching. The egg envelope has external ridges, which are centred around the micropyle. Hatching occurs within 168–204 hours. Exogenous feeding started on day 17 with a total length of 16.2 mm and yolk-sac remnants still present. The larval period lasts until day 36. Dark brown stripes appear on the body as one of the characteristic pigment patterns of juvenile *N. notopterus* at day 70 with a total length of 34 mm. The genital papilla can macroscopically be recognized at day 80. Sexual maturity of *N. notopterus* is first observed in 30-month old specimens with a total length of 275 mm.

For the first time, this study describes a method of successfully raising *O. bicirrhosum* at 28 °C under laboratory conditions. The non-adhesive eggs measure 12 mm with a transparent egg envelope. Hatching occurs around 162–166 hours and newly hatched embryos measure 16 mm. Mixed feeding is observed at the age of 26 days, with the juveniles reaching a total length of 38 mm. Around the age of 100 days and with a total length of 125 mm, the juvenile is similar to an adult.

The embryonic period in *O. bicirrhosum* lasts longer than in *N. notopterus*. Actually there is no larval period found in *O. bicirrhosum*. The embryonic period is directly followed by the juvenile period and ontogeny can be characterized as direct development. *N. notopterus* is classified as intermediate species in an interpretation at reproductive strategies since they produce a higher number of medium-sized eggs and show parental care.

ZUSAMMENFASSUNG

Die Osteoglossomorpha stellen eine basale Gruppe der Teleostei dar mit einer Mischung von plesiomorphen und apomorphen Merkmalen bezogen auf Reproduktion und ontogenetische Entwicklung. Bezüglich reproduktiver Gilden und ontogenetischer Entwicklung gibt es immer noch nur begrenzte Daten zu dieser Gruppe. Einige Ergebnisse zu verschiedenen Aspekten der Reproduktion sind vorhanden. Der größte Teil tiefer gehender Studien bezieht sich auf Mormyriden, detaillierte Beschreibungen und experimentelle Daten sind kaum vorhanden bei den anderen Gruppen sowie bei *Notopterus notopterus* und *Osteoglossum bicirrhosum*.

Im Rahmen dieser Untersuchung wird zum ersten Mal eine detaillierte Beschreibung der ontogenetischen Entwicklung dieser beiden Arten auf der Basis von Laborzuchten vorgestellt. Zuchtexperimente hatten zum Ziel, den möglichen Einfluss von Umweltparametern auf Gonadenentwicklung und Balzverhalten von fünf Arten aus drei Familien der Ordnung der Osteoglossiformes zu untersuchen: Mormyridae (*Petrocephalus soudanensis* und *P. catostoma*), Pantodontidae (*Pantodon buchholzi*) und Notopteridae (*N. notopterus* und *Xenomystus nigri*). Zum Studium der Osteoglossidae (*O. bicirrhosum*) wurde eine fünfmonatige Felduntersuchung in Kolumbien durchgeführt.

Nur bei *N. notopterus* gelangen Zuchtexperimente unter Laborbedingungen. Die experimentellen Daten zeigten, dass die Umweltparameter abnehmende Leitfähigkeit, Erhöhung des Wasserstandes und leichte Temperaturvariation keinen Einfluss auf Gonadenentwicklung oder Balzverhalten bei *N. notopterus* hatten. Das Ablaichen erfolgte zur Tageszeit bei 25-28 °C. Die frisch abgelegten, klebrigen Eier von 3.8-4 mm Größe werden vom Männchen bis zum Schlupf bewacht. Die Eihülle besitzt äußere Rillen, die ringförmig um die Mikropyle herum angeordnet sind. Das Schlüpfen erfolgt im Alter von 168-204 Stunden. Exogene Nahrungsaufnahme begann am 17. Tag bei einer Totallänge von 16.2 mm; zu diesem Zeitpunkt sind noch Reste des Dottersackes vorhanden. Die Larval-Periode dauert bis zum 36. Tag. Dunkelbraune Querstreifen erscheinen auf den Flanken als ein charakteristisches Juvenil-Farbmuster um Tag 70 bei einer Gesamtlänge von 34 mm. Die Genitalpapille ist ab dem 80. Tag makroskopisch sichtbar. Die Geschlechtsreife stellte sich bei 30 Monate alten Tieren ein bei einer Totallänge von 275 mm.

In dieser Studie wird zum ersten Mal eine Methode zur erfolgreichen Aufzucht von *O. bicirrhosum* unter Laborbedingungen bei 28 °C vorgestellt. Die nicht-klebrigen Eier von 12 mm Größe besitzen eine transparente Eihülle. Das Schlüpfen erfolgt im Alter

von 162-166 Stunden und die geschlüpften Embryonen haben eine Länge von 16 mm. Exogene Nahrungsaufnahme beginnt bei gleichzeitigem Vorhandensein von einem großen Dottersack bei einer Gesamtlänge der Juvenilen von 38 mm. Im Alter von etwa 100 Tagen und bei einer Gesamtlänge von ca. 125 mm besitzt der Juvenile schon die Proportionen eines Adulten.

Die Embryonal-Periode bei *O. bicirrhosum* dauert länger als bei *N. notopterus*. Bei *O. bicirrhosum* findet sich keine Larval-Periode; auf die Embryonal-Periode folgt sofort die Juvenil-Periode: Die Ontogenese kann somit als direkte Entwicklung klassifiziert werden. *N. notopterus* hingegen ist gekennzeichnet durch eine intermediäre Entwicklung unter Bezug auf Reproduktionsstrategien da sie eine höhere Anzahl von mittelgroßen Eiern produzieren bei gleichzeitiger Brutpflege.

1 INTRODUCTION

1.1 General overview on Osteoglossomorpha

The Osteoglossomorpha or “bony tongues” comprise a group of morphologically and biologically diverse primitive teleostean fish. The superorder was defined by Greenwood et al. (1966). With a few exceptions, osteoglossomorphs have most of their teeth located on the tongue (osteo, “bony”; glossid, “tongue”) and on the roof of the mouth (or the parasphenoid) (Moyle, 2004). They also have a caudal fin with 16 or fewer branched rays, no intermuscular bones in the back of the body (epipleurals), cycloid scales with ornate microsculpturing, and an intestine that curls around to the left side of the oesophagus rather than to the right as in most other bony fish (Nelson, 1972). Although fossil evidence is sparse, osteoglossomorphs may have formed an important element in the freshwater fauna of the world before the emergence of the ostariophysans.

The Osteoglossomorpha contain two orders, the Osteoglossiformes and the Hiodontiformes. Recent studies (see Li, 1994a; Li and Wilson, 1996a; Li et al., 1997) support the concept of a sister-group relationship between the Hiodontiformes and the Osteoglossiformes. The Osteoglossiformes consist of five families: the Osteoglossidae, Notopteridae, Pantodontidae, Mormyridae, and Gymnarchidae. The Hiodontiformes on the other hand are made up of only one single family, namely the Hiodontidae. The fish of the family Osteoglossidae are found in South America, Africa, Asia, and Australia, the Notopteridae in Africa and South-East Asia; the Mormyridae, Gymnarchidae and Pantodontidae in Africa and the Hiodontidae solely in North America.

The relationship of the osteoglossomorphs to other fish groups is not fully defined, presumably because they are such an ancient group. Greenwood (1973) suggested a sister-group relationship between Osteoglossomorpha and Clupeomorpha, while Patterson and Rosen (1977), Lauder and Liem (1983), and Nelson (1994) considered the Osteoglossomorpha to be the most primitive living teleosts.

The seven species of the family Osteoglossidae are conspicuous members of their local faunas, with heavy and elongate bodies. The dorsal and anal fins are long and placed on the rear half of the body. Family characteristics also include: a scale-less head, a large mouth usually turned upwards, pointed pectorals and small pelvic fins, a small or reduced caudal and a mosaic-like pattern of large bony scales (Sterba, 1973; Greenwood, 1975). Most species are predators and live in tropical rivers. All species of the Osteoglossidae can breathe air through their lung-like swim bladders (Moyle, 2004).

The two species of *Osteoglossum* (*O. bicirrhosum*, *O. ferreirai*) exist in South America. *O. ferreirai* (Black Arowana) can be exclusively found in the Negro River basin, whereas *O. bicirrhosum* (Silver Arowana) inhabit the rest of the Amazon basin, the Orinoco basin and various drainages of the Guyanas (Cala, 1973). The distribution of these two species is based on different water types: *O. ferreirai* mainly occurs in acidic black waters, while *O. bicirrhosum* is found in neutral or even slightly alkaline waters (Saint-Paul et al., 2000). The Silver Arowana can reach up to 120 cm in length (Lüling, 1976). *O. bicirrhosum* is known to be one of the most caught species in the wild in South America, which has an impact on its population size. In Colombia, *O. bicirrhosum* (Silver Arowana) is bred for the ornamental fish market. Australia is the natural habitat of *Scleropages leichhardtii*, *S. formosus* and *S. jardini*. *S. formosus* can be additionally found in South-East Asia and *S. jardini* in New Guinea. *S. leichhardtii* can reach up to 90 cm in length and 4 kg in weight, though it commonly measures around 50 to 60 cm in length (Merrick et al., 1983). The Asian Arowana (*S. formosus*), the most popular yet very expensive species on the aquarium trade market (Fernando et al., 1997), is a large and attractive fish, reaching up to 7 kg in total weight and 100 cm in total length (Alfred, 1964). The Saratoga, also called Spotted Barramundi, *S. jardini*, was reported to attain a maximum length of more than 90 cm and maximum weight of 17.2 kg, though most specimens are 50 to 65 cm long (Anon, 1977). With a maximum length of 300 cm, *Arapaima gigas* is one of the largest freshwater fish of South America. The only member of the Osteoglossidae, that is not predatory but planctivorous, is *Heterotis niloticus* from western Africa.

The family Notopteridae comprises four genera and 10 species with mostly long and notably slender bodies. They are able to swim backwards equally well as forwards. The dorsal fin is small and featherlike, so these fish are commonly referred to as “Feather backs” or “Knife fish”. Knife fish have a long anal fin, which conjoins with the caudal fin and closes behind the ventral fins. The mouth has many small teeth and the body is covered with numerous tiny scales. The swim bladder is connected to the gut and is used for breathing air. This swim bladder may have three functions: as a means for aerial respiration, as an accessory auditory organ, and as an organ for sound production (Greenwood, 1963). The knife fish live in stagnant backwaters and ponds. *Notopterus notopterus* and the large growing species of the genus *Chitala* (*C. chitala* and *C. ornata*) are fish from tropical South-East Asia (Moyle, 2004). Its natural habitats are located in India, Pakistan, Burma, Thailand, the Malay Archipelago, the Philippines, Cambodia, Vietnam, Laos, Bangladesh, and Myanmar (Day, 1889; Roberts, 1992). *N. notopterus* is predominantly a carnivorous and a column-feeder (Mustafa and Ahmed, 1979).

Xenomystus nigri occurs in Africa primarily in Nile, Chad, Niger, and Congo Basin (Nelson, 2006). *X. nigri* can reach up to a maximum length of 22 cm and is adapted for nocturnal life. The dorsal fin absents in this species. Some papers reported that *X. nigri* possesses electroreceptors (Bullock and Northcutt, 1982; Braford, 1982). Other notopterid species, *Papyrocranus afer* and *P. congoensis* also occur in Africa primarily from Senegal to Nigeria and the Congo Basin (Nelson, 2006).

The family Mormyridae is a highly speciose group of African weakly electric fish. According to some authors, it is considered as a separate teleostean order, the Mormyriformes (Scott, 1973; Gosse, 1984; Nelson, 1994; Boden et al., 1997; Alves-Gomes, 1999). They comprise around 200 species (Gosse, 1984) in about 15 genera of mormyrids. They are typically adapted for nocturnal life in large rivers and lakes and are found in all tropical waters. There is a wide range of specialized behaviours and a unique set of anatomical and physiological adaptations associated with the electric sense (electroreception and electrogenesis). These features are believed to be the success indicators of the radiation of this group. The electric system allows them to move at night, to detect prey, and to communicate with each other. Weak electric signals are produced by modified muscles in the caudal peduncle, and an electrical field is set up around each fish (Moller, 1995). Larvae of mormyrids possess a distinct larval electric organ (Kirschbaum, 1977). In addition to the significant electrical system in mormyrids, these fish have a well-developed sense of hearing, which uses the swim bladder to amplify sounds (Werns and Howland, 1976).

Gymnarchus niloticus of the monotypic family Gymnarchidae is closely related to the mormyrids and also possesses an electric system for navigation and detecting prey (Bass, 1982). *G. niloticus* has an elongate body, which it keeps rigid while swimming. It lacks anal, caudal, and pelvic fins, but propels itself with its long dorsal fin, which enables it to swim both forwards and backwards.

The family Pantodontidae is made up of only a single species, *Pantodon buchholzi*, known as “Butterfly fish”. It is a small (10 cm), surface-feeding fish and an obligatory air-breather (Schwartz, 1969). It is most remarkable for its specialized paired fins (Moyle, 2004) and mostly distributed in the freshwaters of West Africa (Teugels, 1990) and the Congo Basin. *P. buchholzi* has been considered as the sister group of the Osteoglossidae (Greenwood et al., 1966; Nelson, 1969; Greenwood, 1973; Taverne, 1979).

The family Hiodontidae has two extant species, the Mooneye (*Hiodon tergisus*) and the Goldeye (*H. alosoides*). They are the most “normal”-looking fish in the entire superorder, since they superficially resemble shad. Their most distinctive external

features are their large eyes with bright gold-silver irises in the Mooneye and gold irises in the Goldeye. The Goldeye inhabits reservoirs, pools and strong currents in larger rivers, and is tolerant of turbidity (Cross, 1967; Pflieger, 1997). This species is known to perform a large-scale migration (Fernet and Smith, 1976). The Mooneye is generally distributed throughout the Great Lakes, but is predominantly found in the waters of Lake Erie in Ohio (Van Oosten, 1961). This species preferably inhabits clear waters and mostly feeds in flowing waters, such as below dams (Trautman, 1957). Both species are carnivorous, feeding on a wide variety of prey, but they are largely piscivorous as adults (Scott and Crossman, 1973).

1.2 Reproduction in Osteoglossomorpha

1.2.1 Influence of environmental factors on gonad development and spawning season

1.2.1.1 Family Osteoglossidae

In all six tropical species of osteoglossid the reproduction is indeed related to the high and low water seasons. Argumedo (2005) reported about the breeding situation of *O. bicirrhosum* in aquaculture, which occurs during rainy season starting in late November and runs until the beginning of July. Between September and October throughout rainy season *S. leichardtii* begins its courting behaviour (Merrick and Green, 1982). Fish spawning in *S. formosus* occurs prior to the onset of the rainy season (Scott and Fuller, 1976; Suleiman, 2003). In contrast, Rowley et al. (2000) stated that the spawning season in *S. formosus* starts at the end of the dry season (March to April) and lasts three months. As for *Arapaima gigas* (Lüling, 1964), reproduction mainly appears to happen during the dry season (low water season).

1.2.1.2 Family Notopteridae

Notopterus notopterus in India mainly spawns during the rainy season with a maximum Gonadosomato Index (GSI) of 6 occurring in June (Parameswaran and Sinha, 1966). This statement was supported by Haniffa et al. (2004): a delay in spawning may occur in India, when the monsoon arrives late. In Bangladesh, spawning in *N. notopterus* commences with the beginning of the rainy season (Azadi et al, 1995). In Cambodia and in the Ganges, *N. notopterus* spawns in the dry season (Mookerjee and Mazumdar, 1946; Chevey, 1941; Southwell and Prashad, 1919). Experimental data by Weitkamp (2005) showed that gonad maturation in *N. notopterus* is independent of conductivity and simulation of rain.

In other notopterids such as *Papyrocronus afer* spawning probably occurs in the rainy season in the swamps of Gambia (Svensonn, 1933); and in the dry season in *Chitala chitala* (Smith, 1933). Experimental data presented by Nyonje (2006) showed that through the imitation of rain, increasing water level, and decrease of conductivity, that gonad maturation in *X. nigri* is independent of three environmental factors aforementioned.

1.2.1.3 Family Mormyridae

There are papers in mormyrids regarding the correlation of reproduction with the alternation of dry and rainy seasons (Kirschbaum, 1975; 1987, 2006; Schugardt and Kirschbaum, 2004, Kirschbaum and Schugardt, 2002; Kirschbaum et al., 2008; Nguyen, 2011). This has been shown experimentally for *Pollimyrus isidori* (Kirschbaum, 1975, 1987); *Mormyrus rume probocirostris* (Schugardt and Kirschbaum, 2004); *Hippopotamyrus pictus*, *Campylomormyrus tamandua*, *Campylomormyrus* sp, *Pollimyrus adpersus*, *Petrocephalus soudanensis* (Kirschbaum and Schugardt, 2001; Kirschbaum, 2006); and *Paramormyrops* of the *magnostipes*-complex (Nguyen, 2011). The rainy season can be imitated (and the gonad development induced) by solely decreasing conductivity.

1.2.1.4 Family Gymnarchidae

Spawning in *Gymnarchus niloticus* probably occurs during the rainy season (Svensson, 1933) and towards the beginning of the flooding season in Senegal and in the Gambia River (Hopkins, 1986).

1.2.1.5 Family Pantodontidae

So far there is only one reference available concerning the influence of environmental factors on the reproduction of *Pantodon buchholzi*. Britz (2004) reported that egg deposition in *P. buchholzi* occurs a few days after a drastic water change (50–80% of tank volume), indicating therefore a correlation between reproduction and the rainy season.

1.2.1.6 Family Hiodontidae

The two species of *Hiodon*: *H. alosoides* and *H. tergisus* spawn during summer (dry season) and spring time (rainy season). According to Battles and Sprules (1960) and Carlander (1969), peak spawning season in *H. alosoides* occurs at the beginning of the dry season (late May to early July). In contrast, Cross and Collins (1995) mentioned that spawning season in this species occurs during rainy season probably in March and April.

In *H. tergisus* spawning occurs in early June and July during the dry season (Fish, 1932). This stands in contrast to what Clark (1979) reported, namely that the peak season of spawning in *H. tergisus* was during the rainy season around the middle of March to April. Also Johnson (1951) stated that spawning in this species occurred in May and April during rainy season. Since hiodontids inhabit temperate zone and are not tropical fish, the influence of environmental factors on gonad maturation remains unclear up to now.

1.2.2 Breeding behaviour

1.2.2.1 Family Osteoglossidae

The five *Scleropages* and *Osteoglossum* species are mouthbrooders and do not show sexual dimorphism (Smith, 1931; Schaller and Dorn, 1971; Merick and Green, 1982; Dawes et al., 1999; Scott and Fuller, 1976). Mouthbreeders in most of the mentioned species in the Osteoglossidae are performed by the male (Argumedo, 2005; Merrick and Green, 1982; Azuma, 1992; Brown, 1995; Schaller and Dorn, 1971; Takeshita, 1973; Scott and Fuller, 1999; Dawes et al., 1999). Nevertheless, two papers also reported that the female of *Scleropages* spp (Schaefer, 2010) and female of *S. leichardtii* (Merrick and Schmida, 1984) are mouthbreeders.

In *S. leichardtii* spawning occurs at night in small ponds during spring time. The eggs of *S. leichardtii* are around 10 mm in diameter (Lake and Midgley, 1970) and vary in number from 30–130 per spawning (Allen et al., 2002). In *S. formosus* spawning occurs in the afternoon (Azuma, 1992). Females of *S. formosus* produce large eggs around 30-100 (Scott and Fuller, 1976; Dawes et al., 1999) measuring 16-18 mm (Azuma, 1992). During mouthbrooding some eggs maybe dead and embryos are perhaps accidentally released from the parent's mouth. This relates to the paper of Rowley et al., (2000) who reported that a male of *S. formosus* in Cambodia may carry up to 35 juveniles in his mouth.

In *O. bicirrhosum*, the onset of reproduction is indicated by the formation of a pair, which gradually isolates from the group, establishes and defends a sector of the pond as its territory (Argumedo, 2005). Wolfsheimer (1964) documented the large and yolky eggs of *O. bicirrhosum*, which are 16 mm in size; eggs range from 40 (Ungar, 1993) up to 150 (Maupin, 1967; Schaller and Dorn, 1971) per spawning. The eggs of *O. bicirrhosum* after fertilization are reported to be 0.95 g in total weight and approximate 13 mm in diameter (Aragao, 1981). Males of *O. bicirrhosum* and *O. ferreirai* carry the offspring for three weeks and they do not feed at all during this

mouthbrooding phase (Schaller and Dorn, 1971). Sexual maturity starts for the first time at an age of 30 to 36 months in *O. ferreirai* (Argumedo, 2009).

Heterotis niloticus (Budgett, 1901a; Svensson, 1933; Johnels, 1954; Daget, 1957) builds nests and guards its eggs and young (Daget, 1957; Moreau, 1974). *Arapaima gigas* also build nests and guard their eggs and young (Fontanele, 1948; Neves, 1998). In both mentioned species, it is still unclear which sex guards the fry.

1.2.2.2 Family Notopteridae

Courtship and spawning activity in *N. notopterus* was observed during the day at a temperature of 26-28 °C. This may last for about seven days (Frieze, 1980). In contrast, Pinxteren (1974) reported that spawning activity occurs during night and females lay eggs on the ground and on stones in water temperature of 25 °C. The eggs are attached to a substrate (Smith, 1933; Axelrod and Burgess, 1981; Frieze, 1980). The 3–4 mm eggs hatched within 11 days according to Axelrod and Burgess (1981), whereas Frieze (1980) mentioned that the eggs are 3.5 mm and hatched after six days at 28 °C. Svensson (1933) reported that newly spawned eggs of *N. notopterus* measured 4 mm. Among Notopteridae, *N. notopterus* and the *Chitala* species perform parental care. The male of *N. notopterus* and *N. chitala* guards the freshly spawned eggs (Pinxteren, 1974; Smith, 1933; Axelrod and Burgess, 1981; Frieze, 1980). *X. nigri* does not perform parental care (Trittelvitz, 1986; Siraad, 1999).

1.2.2.3 Family Mormyridae

Sexual dimorphism becomes apparent at the anal fin in all investigated mormyrids, such as *Petrocephalus soudanensis* (Kirschbaum, 2006); *Pollimyrus isidori* (Kirschbaum, 1987), *P. adspersus* (Kirschbaum and Schugardt, 2006), and *Mormyrus rume probocirostris* (Schugardt and Kirschbaum, 2004). Only *Pollimyrus* (Kirschbaum, 1987; Diedhiou et al., 2007a) and *Stomatorhinus* (Heymer and Harder, 1975) show parental care in mormyrid species.

Spawning in mormyrids occurs mainly at night as seen in *Campylomormyrus cassaicus*, *Hippopotamyrus pictus*, *Mormyrus rume probocirostris*, *Pollimyrus isidori* and *P. adspersus* and *Petrocephalus soudanensis* (Kirschbaum, 1987, 2006; Kirschbaum and Schugardt, 2002; Diedhiou et al., 2007a). Nguyen (2011) mentioned that spawning after hormonal injection occurs at first during daylight in the fishes of *Paramormyrops magnostipes*-complex. Spawning site preference exists in *Mormyrus rume probocirostris*, *Campylomormyrus cassaicus*, *Hippopotamyrus pictus* (Kirschbaum and Schugardt, 2004), and in the fishes of *Paramormyrops magnostipes*-complex (Nguyen, 2011; Arnegard et al., 2005).

1.2.2.4 Family Gymnarchidae

Gymnarchus niloticus builds large floating nests (Budgett, 1901a; Svensson, 1933); containing around 1000 eggs with 10 mm in diameter (Budgett, 1901b). The embryos hatched five days after spawning. Parental care is performed by the male.

1.2.2.5 Family Pantodontidae

The sex of *Pantodon buchholzi* can be determined by the anal fin and by the putative copulatory organ (van Deurs and Lastein, 1973; van Deurs, 1973, 1975). This species was assumed to possess an internal fertilization (Steche, 1915). Spawned eggs are spherical, translucent and floating on the water surface due to large oil globules (Britz, 2004). Eggs diameter measured 2.2–2.4 mm and free embryos hatched on the third day at a temperature of 29 °C after egg deposition (Britz, 2004). This single species exhibits no parental care (Mohn, 1976a; Britz, 2004).

1.2.2.6 Family Hiodontidae

Males of *Hiodon alosoides* and *H. tergisus* have an anal fin with a convex anterior of anal fin, while in females the anal fin is slightly concave (Scott and Crossman, 1973; Hilton, 2002). Both species *Hiodon alosoides* (Battle and Sprules, 1960) and *H. tergisus* (Synder and Douglas, 1978) do not exhibit parental care.

Sexual maturity in *H. alosoides* may occur as early as age 1 year (Synder and Douglas, 1978). Spawning is assumed to take place at night (Scott and Crossman, 1973). Eggs deposited on rocks (Scott and Crossman, 1973) and gravel (Balon, 1975a). Eggs are spherical, buoyant, non-adhesive with a single large oil globule, measuring 4 mm in diameter (Fish, 1932). Hatching occurs in about 14 weeks (Scott and Crossman, 1973).

Sexual maturity in *H. tergisus* usually reached in third or fourth year at 228-280 mm (Van Oosten, 1961). Eggs deposited over rocks and gravel (Balon, 1975b). Females produce around 3000-7.700 eggs in Tennessee River (Wallus and Buchanan, 1989). Fertilized eggs are spherical, non-adhesive and buoyant (Balon, 1975b).

1.2.3 Early ontogeny and objectives of the study

There are a number of papers concerning the early development in Osteoglossomorpha. Argumedo (2005) has written a handbook on commercial breeding in aquaculture of *Osteoglossum bicirrhosum* including a staging of development. Wolfsheimer (1964) and Ungar (1993) reported briefly on the development of *O. bicirrhosum*. In general, information on the early ontogeny of *O. bicirrhosum* is still very scarce.

Regarding notopterids, a recent study by Srivastava et al. (2012) described the embryonic and larval development in fixed specimens of *Notopterus notopterus*. However a huge gap remains concerning the detailed description of the egg and the egg envelope, early cleavage, larval and juvenile development.

Regarding mormyrid species, the ontogeny of several species has been described, e.g. in *Hyperopisus bebe* (Johnels, 1954), *Pollimyrus adspersus* (Kirschbaum, 1987), *Mormyrus rume probosciostris* (Kirschbaum and Schugardt, 1995; Schugardt and Kirschbaum, 1996, 2004, 2006), *Campylomormyrus tamandua* (Schugardt and Kirschbaum, 1998), *Hippopotamyrus pictus* (Kirschbaum and Schugardt, 2002b), and *Petrocephalus soudanensis* (Kirschbaum, 2006). Yet only Diedhiou et al., (2007b) documented for the first time in detail the ontogenetic development in the mormyrid *Pollimyrus isidori* and proposed a staging system.

The early ontogenetic development in *Gymnarchus niloticus* has been described by Budgett (1901), Assheton (1907) and Svensson (1933). However detailed information on the early ontogeny of *G. niloticus* remains limited.

In *Pantodon buchholzi*, Britz (2004) described the newly spawned egg, its structure, the newly hatched embryo and the larval development. He also summarized characteristics of reproductive styles in 16 additional species of osteoglossomorphs.

In hiodontid species, Battle and Sprules (1960) published a description of the free embryo stage and the larval stage in *Hiodon alosoides*. Synder and Douglas (1978) presented a very short description of the free embryonic stage in *H. tergisus*. A summary of morphometric data for early life phases of *H. tergisus* is described by Wallus and Buchanan (1989).

This study will fill some gaps on early ontogenetic development and will present for the first time a detailed ontogeny of *Notopterus notopterus* (Notopteridae) and *Osteoglossum bicirrhosum* (Osteoglossidae) together with experimental data of two mormyrid species *Petrocephalus soudanensis* and *P. catostoma*, and *Pantodon buchholzi* (Pantodontidae) and *Xenomystus nigri* (Notopteridae). Therefore, the objectives of the present study are: 1) to present a detailed description of ontogenetic development in *N. notopterus* and *O. bicirrhosum*, and 2) to describe and to evaluate the characteristic differences between these two species: the substrate spawner *N. notopterus* and the mouth breeder *O. bicirrhosum*. The study aims at a more detailed comparison with other osteoglossomorphs in view of elucidating a bit more the heterogeneous systematic distribution of different reproductive styles among Osteoglossomorpha.

2 MATERIALS AND METHODS

2.1 Mormyridae

2.1.1 *Petrocephalus soudanensis*

The four specimens (3♂, 1♀) of *Petrocephalus soudanensis* (**Fig. 1**), which were used for the first breeding experiment, were already present at the Humboldt University before the start of this project. They were kept in a 155 x 62 x 50 cm tank (500–600 l) equipped with a biofilter and PVC tubes as hiding places (**Fig. 2**). Conductivity was decreased by continuously adding deionised water of a conductivity of 25 $\mu\text{S}/\text{cm}$. Temperature and conductivity were measured daily by using a conductivity meter from WTW Tetracon 325. The fish were fed with *Chaoborus* sp. twice a day.



Fig. 1. A male *Petrocephalus soudanensis* with a total length of 10.6 cm [a]. [b] The base line of the anal fin (arrowhead) of the male is concave and has longer fin rays than [c] the female's, whose base line of the anal fin is straight. Scale bar = 3 cm.

2.1.2 *Petrocephalus catostoma*

Seven specimens (6♂, 1♀) of *Petrocephalus catostoma* (**Fig. 3**) were donated by Prof. Dr. B. Kramer. The fish originated from the Namibia-East Caprivi upper Zambezi River at Katima Mulilo, West Africa. The fish were kept in tanks similar to those of

P. soudanensis and the breeding experiment was performed in a similar way. During the experiment, all tubes in the breeding tank were completely infested with planarians. The tubes were used as hiding places. Therefore it was necessary to wash out the tank with hot water twice a week.



Fig. 2. The experimental tank of *Petrocephalus soudanensis* equipped with plastic tubes (x) covered by a plastic sheet (arrow).



Fig. 3. A male *Petrocephalus catostoma* with a total length of 10.9 cm. Scale bar = 3 cm.

2.2 Pantodontidae

2.2.1 *Pantodon buchholzi*

All specimens of the Butterfly fish *Pantodon buchholzi* (**Fig. 4**), originating from the Niger, West Africa, were purchased from a whole sale dealer (Aquarium Glaser in Rodgau, Frankfurt am Main). The first group of *Pantodon* (n=16) arrived on 5 February 2009, consisting of 11 males and 5 females. Total length ranged from 75-107 mm and total weight from 3.74–9.93 g. The second group arrived on 6 May 2009, comprising 10 fish (9♀, 1♂), their total length ranging from 77–105 mm and their total weight from

2.45–6.94 g. The third group (n=100) was purchased on 10 December 2010. Due to illness, 85 specimens died in the tank a week upon arrival. Only 15 specimens (5♀, 10♂) survived.

The sex of a butterfly fish can be clearly determined by the shape of its anal fin. The female's fin has a straight line at the rear edge. The male anal fin on the other hand is notched or indented and almost looks like two fins (**Fig. 4a, b**), with a short and straight posterior and long and filamentous anterior part (Vriends, 1978). They were fed on crickets (*Acheta domesticus*) twice a day, *ad libidum*.

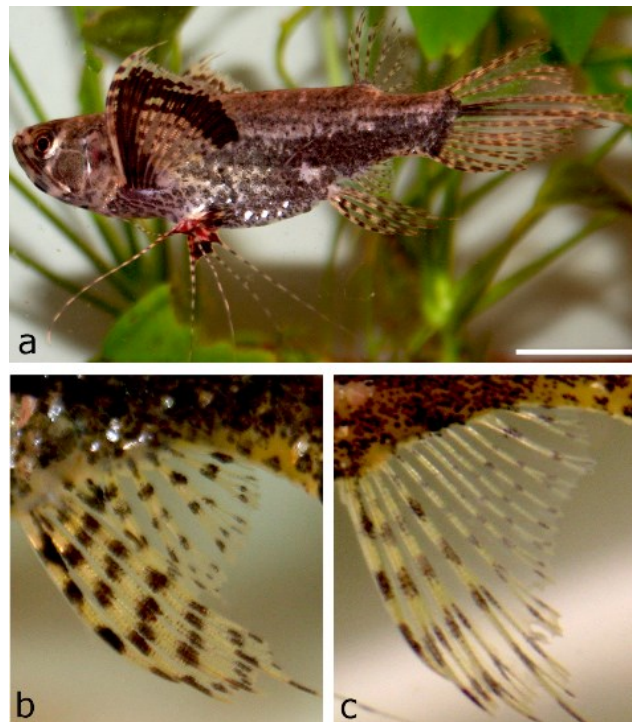


Fig. 4. A male *Pantodon buchholzi* with a total length of 9.6 cm [**a**]. The anal fin of a male [**b**] is notched and a female's is straight [**c**]. Scale bar = 2 cm.

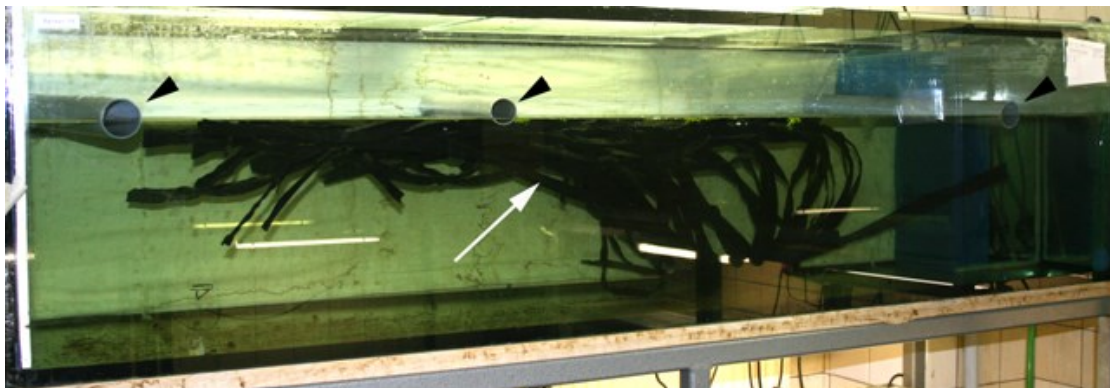


Fig. 5. The experimental tank of the first group of *Pantodon buchholzi* equipped with black polythene shreds (arrow) as plant imitation. The three small plastic tubes (arrowhead) were placed on the surface of the water to prevent the crickets, which were fed to the fish, from leaving the tank.

The three groups of fish were put into three separate tanks: group one in a 200 x 60 x 50 cm tank 600 l, equipped with black polythene shreds as plant imitation (**Fig. 5**), the second group in a 150 x 60 x 50 cm tank (450 l), and the last group in a smaller 90 x 60 x 53 cm tank (300l). Conductivity and temperature were measured daily.

2.3 Notopteridae

2.3.1 *Notopterus notopterus*

2.3.1.1 Specimens and breeding tanks

Some specimens of *Notopterus notopterus* (**Fig. 6**), originating from South-East Asia, had already been present at the Humboldt University of Berlin, before the onset of these investigations. These were nine females and five males with a total length of 198–269 mm and a total weight of 45.97–158.66 g. The additional 20 specimens (10♀, 10♂) were purchased from a wholesale dealer (Aquarium Glaser in Rodgau, near Frankfurt am Main). This group consisted of individuals ranging from 178–248 mm in total length and 39.38–136.77 g in total weight.

Sexes of *Notopterus notopterus* can be distinguished by the shape of the genital papillae (Weitkamp, 2005). The male's narrow genital papilla is of reddish color and longer than the rudimentary pelvic fin, while the female's broader one is of whitish color and shorter than its pelvic fin (**Fig. 7**). The fish were fed once a day on beef heart and/or frozen chironomids.



Fig. 6. A female *Notopterus notopterus* with a total length of 23 cm. Scale bar = 2 cm.

Two tanks, one of 700 l and 400 l volume, respectively, were used to breed this species with sex ratios 2:1 and 1:1 (female/male). Each tank had been disinfected before usage. One breeding tank was equipped with black polythene shreds as plant imitation or hiding places and some flat large stones as spawning substrates (**Fig. 7a**), whereas the

other tank was supplied with two large stones and sand, covering the entire bottom of the tank (**Fig. 7b**).

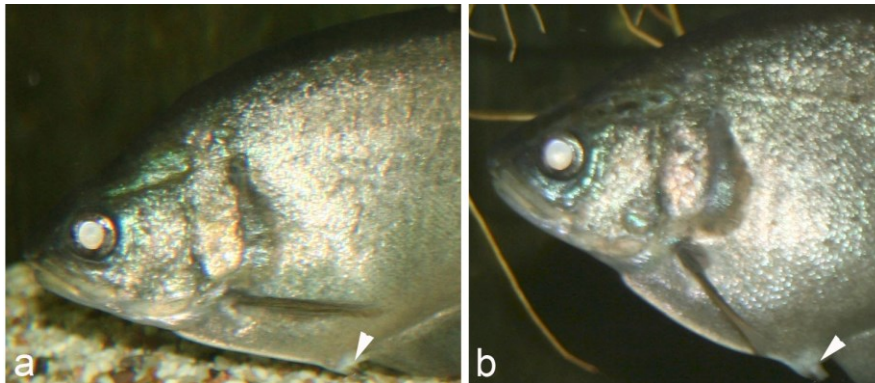


Fig. 7. Adult male [a] and female [b] *Notopterus notopterus*; the genital papilla (arrowhead)

The acclimatization period lasted about six months. This period of time was used to observe the selected specimens for breeding purposes and also to create the desired breeding conditions. During this phase, the fish established individual territories. Moreover, the male chose a female partner for courtship and mating.

2.3.1.2 Rearing

Eggs, embryos and larvae were attained from ten spawning occurrences. Directly after each spawning, the eggs were removed from the breeding tank and transferred into 20 x 10 x 6 cm plastic jars covered with a plastic lid or into Petri dishes measuring 5-10 cm in diameter.



Fig. 8. Two experimental tanks of *Notopterus notopterus*. [a] A tank equipped with flat stones (x) and black polythene shreds (arrow). [b] The tank was filled up with sand on the ground and equipped with two large stones (x) as well as live plants and roots of *Monstera*.

They were afterwards placed in a thermostat (27 °C, no aeration system) until the larvae started exogenous feeding. Larvae were fed for the first 7 days with fresh, newly hatched *Artemia* nauplii. Subsequently on the eighth day of feeding, food supply was substituted by older *Artemia* nauplii supplemented with small pieces of *Tubifex*. As the

larvae grew, they were transferred into a small tank (20 x 20 x 20 cm) and provided with small pipes as hiding places. Once older than three months, the juvenile fish were fed with small pieces of beef heart twice a week.

2.3.2 *Xenomystus nigri*

2.3.2.1 Specimens and breeding tanks

The first 16 (9♀, 7♂) fish arrived on 30 March 2009 and comprised a range in total length of 85–120 mm (**Fig. 9**). The fish, originating from the Niger Basin, were purchased from a whole sale dealer. This group was placed in a tank measuring 120 x 75 x 60 cm and one third of the water was regularly changed once a week. The first tank was furnished with black polythene shreds as hiding substrates.

Two weeks upon arrival, some of the fish were infected with the ectoparasite *Ichthyophthirius* known as white spot disease. Medicinal treatment was performed for three weeks, although three heavily infected fish died. The remaining fish recovered well. Acclimatization was continued in a disinfected tank for about 60 days.



Fig. 9. Adult female *Xenomystus nigri* with a total length of 10.7 cm. White arrowhead points to the swollen belly. Scale bar = 2 cm.

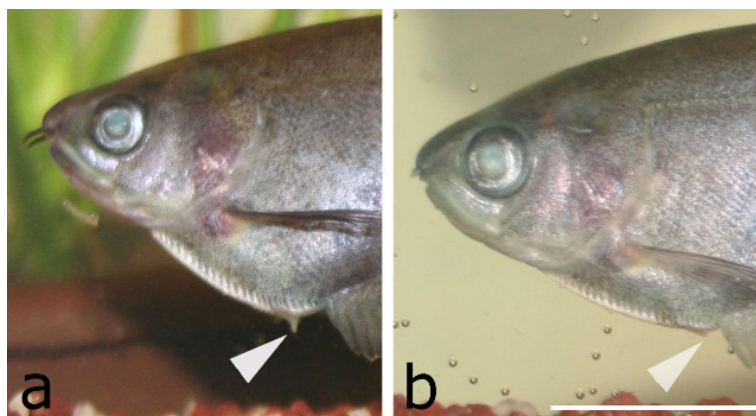


Fig. 10. The genital papillae in *Xenomystus nigri* can be used for the differentiation between [a] female and [b] male; the male has a short protruding papilla (arrowhead) lying underneath the pelvic fin while the female's genital papilla is longer. Scale bar = 1 cm.

The second group was purchased on 6 May 2009 from a wholesale dealer (Aquarium Glaser in Rodgau near Frankfurt am Main) consisting of 10 specimens (9♀, 1♂) with a total length of 128–173 mm. The specimens were placed into a tank measuring 90 x 60 x 53 cm, which was also supplied with black polythene shreds and two PVC tubes as hiding places. Acclimatization period lasted about three months. *Xenomystus nigri* has no sexual dimorphism, except for the genital papillae, which is remarkably longer than the pelvic fin in females, while the male's papilla is a short protrusion lying underneath the pelvic fin (Nyonje, 2006) (**Fig. 10**). Fish were fed once daily with live *Chaoborus* sp. and/or frozen chironomids. Selected specimens with swollen bellies received several hormonal injections (**see 2.8**). Three tanks (90 x 60 x 53 cm) were furnished with several PVC tubes, stones and woods. Each of tanks comprised one male and female. The selected specimens were separated for two weeks before the injection. Immediately after the injection, all specimens were returned into their previous tanks.

2.4 Osteoglossidae

2.4.1 *Osteoglossum bicirrhosum*

2.4.1.1 Location and fishes

Study of the Silver Arowana (*Osteoglossum bicirrhosum*) (**Fig. 11a**) took place in Florencia, Southeast Colombia for around 19 weeks. The samples were collected from different ponds and/farms located at least 5 km outside of the city (El Doncello, El Paujil, Montañita, Morelia, San Jose, San Vicente). Samples consisted of eggs and juveniles of *Osteoglossum bicirrhosum* in different stages of development. *O. bicirrhosum* can be kept together with different fish species such as *Oreochromis niloticus* (family Cichlidae), *Arapaima gigas* (family Osteoglossidae), *Piaractus brachypomus* (family Serrasalminidae), *Colossoma macropomum* (family Characidae), *Potamotrygon* sp. (family Potamotrygonidae) and *Pseudoplatystoma fasciatum* (family Pimelodidae). The fish were fed with pellets and live guppies, *ad libidum*. Since tap water could not be utilized due to the high level of Chlorine (CL), water taken directly from the river nearby was used. The water temperature ranged from 26–31 °C depending on weather conditions and pH value varied from 6.8–7.2. About one meter above the surface the ponds were covered with nets for protection from predators.

2.4.1.2 Collection of samples

Prior to collecting the samples, some observations of fish behaviour had to take place. Guarding males ignored the pellets, as they cannot swallow them. Every specimen of

Silver Arowana with a closed mouth and swollen hard jaw contained eggs, embryos, or even free embryos. The volume of the ponds (**Fig. 11b**) ranged from 600 to 3000 cbm. Collecting the samples always took place either in the early morning or late afternoon in order to avoid the strong sun.

The heavy rainy season is used as a signal for the start of the spawning season of *Osteoglossum* ssp by the farmers. Using a long net (ca. 45 m long) the fish were driven to the edge of the pond (**Fig. 11c**). The selected specimen was gently touched on the bottom of the jaw to stimulate the opening of its mouth. The eggs or embryos found in its mouth were cautiously poured into a plastic jar filled with water from the pond (**Fig. 11d**) and shielded from direct sunlight. Subsequently they were carefully put into a transparent plastic sac filled with clean water of the breeding pond and supplied with oxygen (**Fig. 11e**). Eggs and embryos were kept separately.

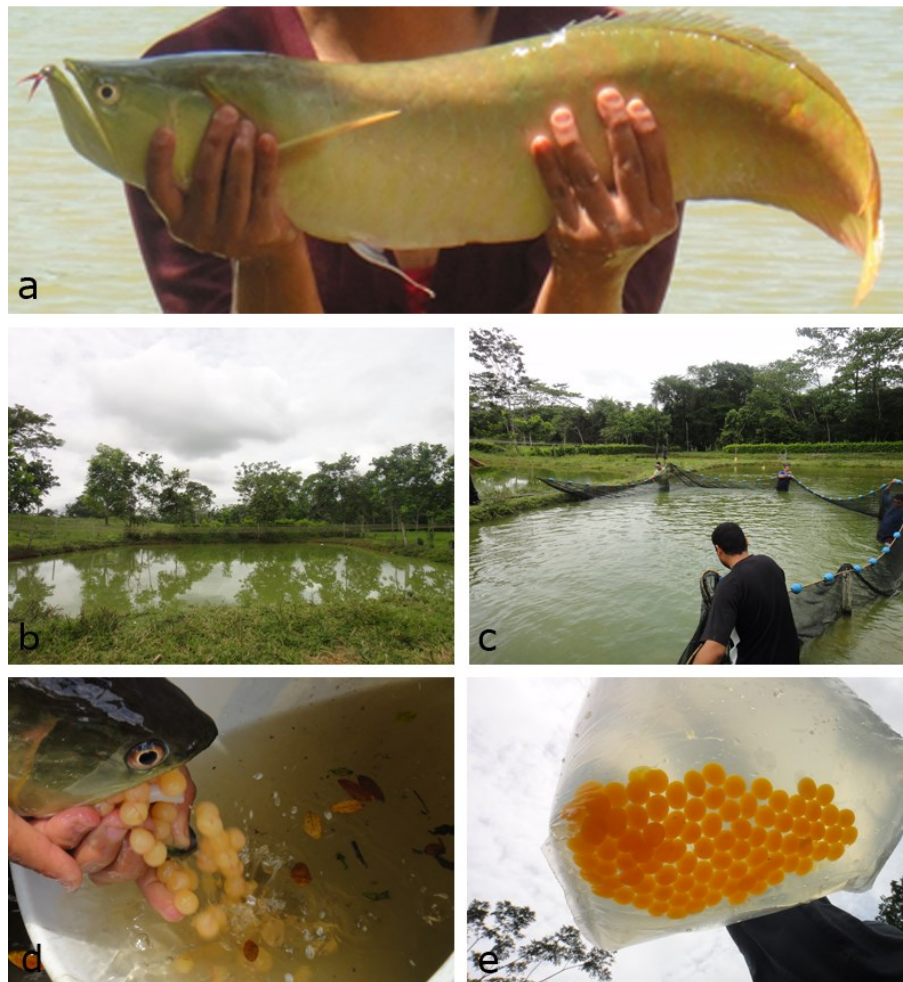


Fig. 11. Collecting samples on the farm. [a] Adult specimen of *Osteoglossum bicirrhosum* with a total length of 73 cm and 4 years old, located in Paujil. [b] This pond contained 40 Silver Arowana and was covered with a net for protection from predators. [c] By using a long net pulled by hand, all fish were gathered to the edge of the pond. [d] Transferring the eggs into a plastic jar. [e] Collected eggs were put into a transparent plastic bag ready to be transported to the laboratory.

They were directly transported to the city for further investigation. The transport itself took around 2–3 hours. Unfertilized eggs or dead specimens (recognized by white colouring and gradually damaging process of the egg envelope) were immediately removed from the remaining specimens (**Fig. 12**).

2.4.1.3 Rearing and documentation

To raise the eggs (**Fig. 13a**) and embryos (**Fig. 13b**), the samples were respectively put into two separate aquaria (25 x 15 x 20 cm) (**Fig. 13**). Each aquarium was equipped with a filter, heater, thermometer and a lid made from Styrofoam. Especially the freshly captured eggs were put into a small transparent glass container inside the aquarium that was equipped with two filter devices: one to produce the oxygen and the other, which was connected with a tiny tube, to produce a slight current for maintaining a continuous movement of the eggs (**Fig. 13a, b**).

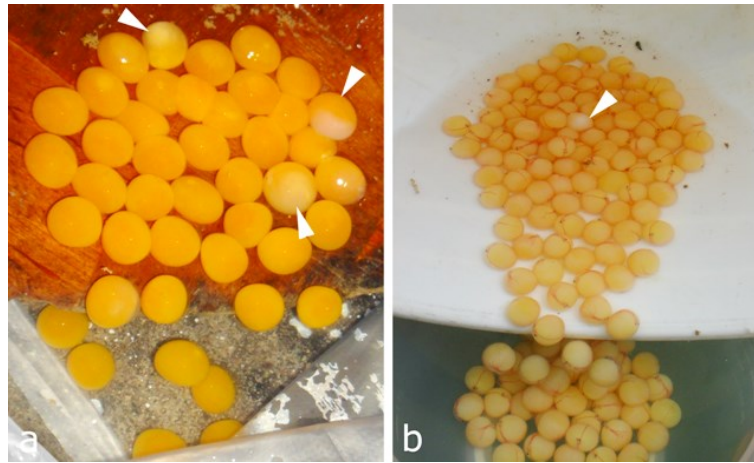


Fig. 12. Separation of samples in *Osteoglossum bicirrhosum*. [a] Freshly collected eggs and [b] embryos were directly separated from the unfertilized eggs which can be recognized by their white colour (arrows); these pictures were taken from two different farms.

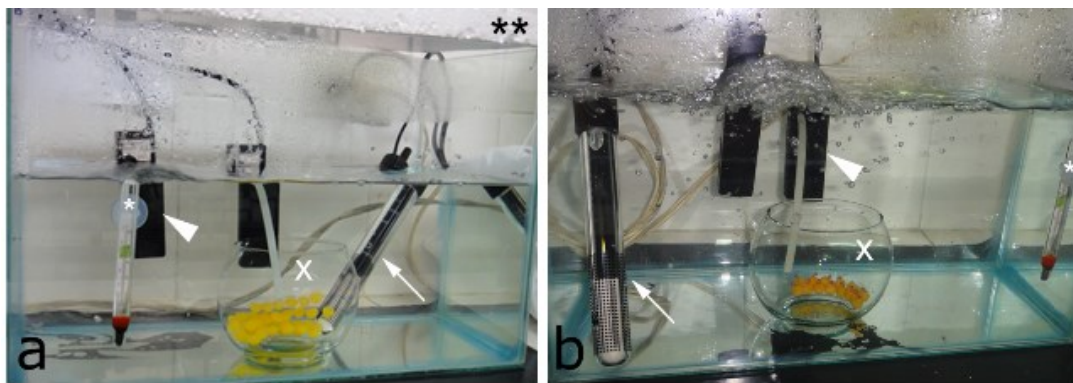


Fig. 13. Aquarium set up containing [a] freshly collected eggs and [b] embryos of *Osteoglossum bicirrhosum*. Transparent glass container (x), heater (arrow), thermometer (*), two filter devices (arrowhead) and a Styrofoam lid (**) were used to maintain stable conditions for raising the eggs and embryos.

Water used for the aquaria, taken from the local farm, was regularly changed to uphold its quality. The water temperature in the aquarium was kept constant at ca. 28 °C with pH ranging from 6.8–7.2; water level varied from 15–18 cm. Exogenous feeding commenced earlier than expected even though the yolk sac had not been resorbed yet. During this period the embryos were fed three times a week with flakes. Once the yolk sac had been completely resorbed, the juveniles kept on being fed twice a day with flakes and/or substituted with small guppies, *ad libidum*.

On 22 February 2011, 16 dead specimens were collected from a nearby pond consisting of two specimens of *Osteoglossum bicirrhosum* and 14 specimens of *Osteoglossum ferreirai*. They were measured and dissected two hours after having been taken out of the pond. The time delay was due to the transport from the farm to the laboratory. *In vivo* observations on freshly captured eggs and embryos were photographed by using a stereomicroscope Nikon SMZ 7454 connected with Optika Vision Pro software. Meanwhile photos of the development of embryos, juvenile and adult were captured by using Sony TX7.

2.5 Variation and measurement of environmental parameters

Variation of environmental parameters to induce the gonad maturation was stimulated with the method developed by Schugardt and Kirschbaum (2004), namely through manipulation of the conductivity of the water. Continuous decreasing conductivity was achieved by adding demineralised water to the water in the experimental tanks. The water level (WL) was kept constant through an overflow system. Conductivity (C) and temperature (T) were measured daily by using a conductivity meter (WTW Tetracon 325); photoperiod was set at 12L:12D.

2.6 Behaviours

Observation of social, courtship and spawning behaviors was performed during day time. If courtship was intensely seen, evening and night observations were necessary.

2.7 Maturity coefficient (MC)

The gonad's maturity coefficient (MC) of dissected-specimens measured in percentage: (gonad weight/ total weight-total gonad weight) x 100, was used to verify and evaluate the effect of environmental factors on gonad development.

2.8 Hormonal treatment

Some trials of hormonal treatment were applied during the experimental period to a number of specimens of *Xenomystus nigri*. The selected specimens had swollen bellies and showed courtship behaviour regularly for several weeks. They were isolated into a tank of 90 x 60 x 53 cm. In order to obtain ovulated eggs and mature sperm, the selected females and males were injected one after another by using ovaprim (Vancouver, Canada), a GnRH analogue combined with domperidone (a dopamine antagonist). Mature looking females and males (with large abdomen) were anaesthetised with ethyleneglycol monophenyl ether. The anaesthesia was started with the males. Two doses of the GnRH hormone were injected intramuscularly at 50 µg/kg for female with 8–10 g in body weight. Three units of hormone were injected at 75 µg/kg for male with body weight of 10 g. Total weight and total length of each specimen were documented and injection was applied on the left side of the body into the dorsal musculature. After hormone injections the females and males were returned into the same tanks. Hand stripping was applied to the same specimens, if no spawning occurred on the following day. Its abdomen was kept dry and hand stripped applied gently on the area of abdomen.

2.9 General photography

All fertilized eggs, development stages of the embryo and larval development were made with two different cameras: a Leica S6E binocular fitted with a Canon PC1048 micro-camera and a Canon Powershot S50, digital camera mounted on a Leica L2 Stereomicroscope. All pictures taken from the laboratory showing the representative juvenile and adult fish as well as their tanks and *in situ* condition were photographed by using a Canon EOS 350D digital Camera. Photographs were mostly taken of anaesthetised specimens.

2.10 Terminology

The terminology of early ontogeny which was applied here is based on the concept of Balon (1975).

3 RESULTS

3.1 Family Mormyridae

3.1.1 *Petrocephalus soudanensis*

The breeding experiment with *Petrocephalus soudanensis* lasted 371 days (**Fig. 14**). Conductivity was decreased three times throughout the breeding experiment with the first period starting from 804 ($\mu\text{S}/\text{cm}$, day 1) to 114 ($\mu\text{S}/\text{cm}$, day 80), imitating the rainy season to provoke gonad maturation. The conductivity was gradually increased up to 700 $\mu\text{S}/\text{cm}$ (day 117). The second period was started on day 118 and lasted until day 175 with minimum conductivity of 109 $\mu\text{S}/\text{cm}$. The conductivity was increased again steadily for a longer period of time from day 176 to day 294 at 725 $\mu\text{S}/\text{cm}$. The last experimental period started on day 295 and within 40 days there was significantly decreased conductivity to 89 $\mu\text{S}/\text{cm}$, which was followed by a drastically increasing conductivity from 141 ($\mu\text{S}/\text{cm}$, day 351) to 750 $\mu\text{S}/\text{cm}$ (day 353) then again slowly continued increasing to 788 $\mu\text{S}/\text{cm}$. Water level was kept constant at 41 cm and the temperature varied from 24.5 to 27.5 °C.

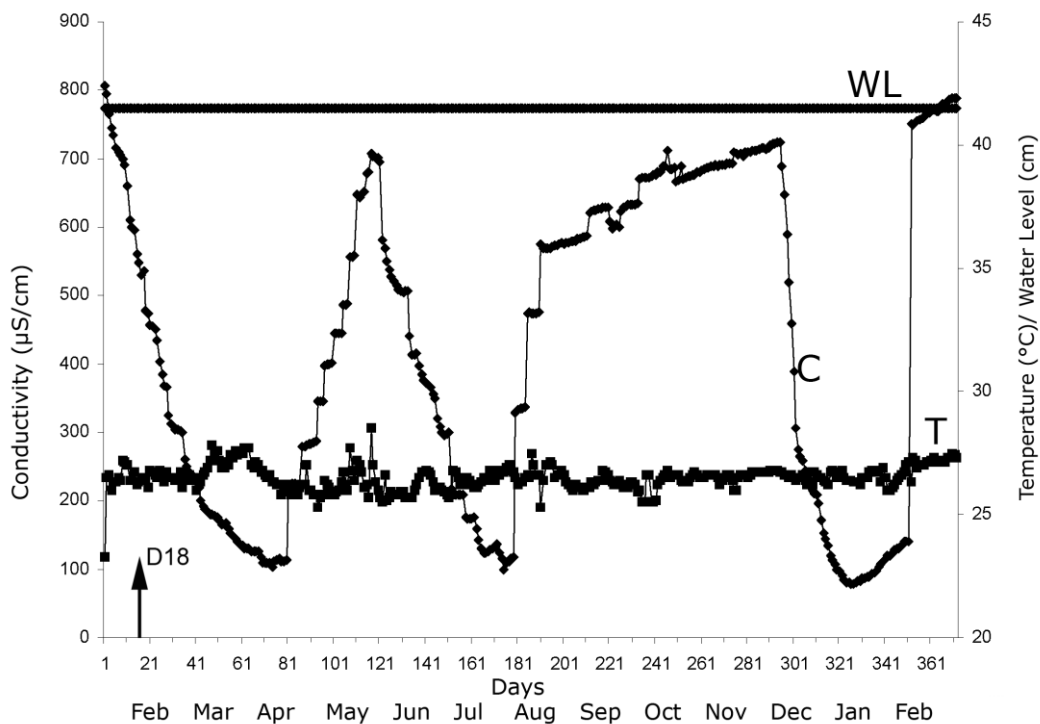


Fig. 14. Course of environmental factors, decreasing conductivity (C), constant water level (WL), and temperature (T) in the breeding experiment with 1 female and 3 males of *Petrocephalus soudanensis* to provoke gonadal recrudescence and spawning. Around day 18 the female showed a swollen belly as indication for gonad maturation. Courtship behaviour was seen, however spawning was not.

Around day 18, the female showed for the first time a swollen abdomen indicating gonad maturation. This followed by intense courtship behaviour five days after (day 23) and lasted for 53 days; a pair was actively seen together during day and night time. Courtship behaviour discontinued during the increasing conductivity. The second swollen belly appeared on day 126 after the onset of the second rainy season and followed by courtship behaviour on day 127 to day 173. On day 183 the female showed a regressed abdomen concomitantly by increasing conductivity. Later on, the belly was seen starting to swell up on day 316 and continuing to do so until day 331, on which a really huge belly was seen, but, unlike the previous symptom, was not followed by courtship behaviour this time. However, even though some intense swollen bellies and courtship behaviour occurred (**Fig. 15, 16**), still no spawning took place.

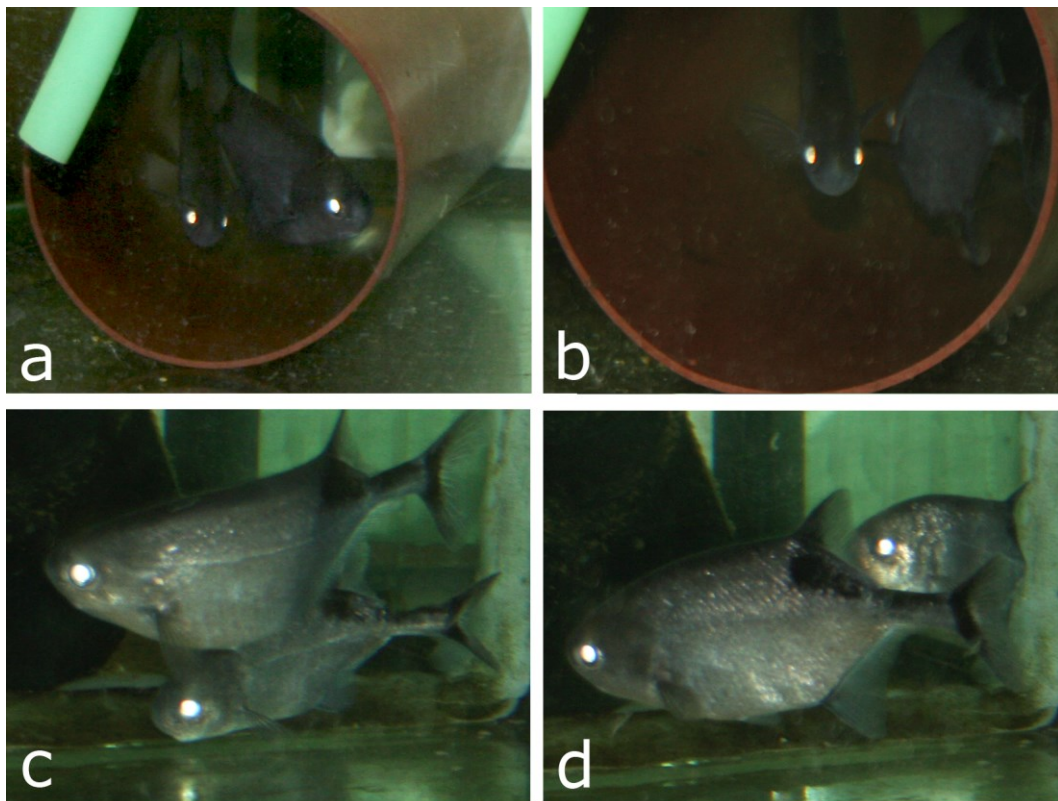


Fig. 15. Courtship behaviour seen in *Petrocephalus soudanensis* during the breeding experiment. [a] A pair was found daily inside a PVC tube on the left side of the tank. [b] They swam and nudged together through the tube. Some actions were captured in the afternoon during courtship behaviour; [c-d] the pair was circling around and swimming alongside for a while on the right side of the tank before returning to the isolated plastic tube on the left side of the tank. The swollen abdomen of the female is visible.

All observed courtship was seen in the morning or prior midday and in the afternoon. The female stayed with one male during the whole courtship period. They were found inside of a PVC tube on the left side of the tank (**Fig. 15a**). They swam and nudged together through the tube (**Fig. 15b**). Once the female left the tube and swam to the right side of the tank, the male directly followed the female, either from behind or from

the other way around. They were circling around and swimming alongside for a while in this area (**Fig. 15c-d**), and then they returned again to the same PVC tube. The pair established a territorial behaviour where they mostly occupied the bottom area around the plastic tubes. The two other males were not involved in courtship behaviour.

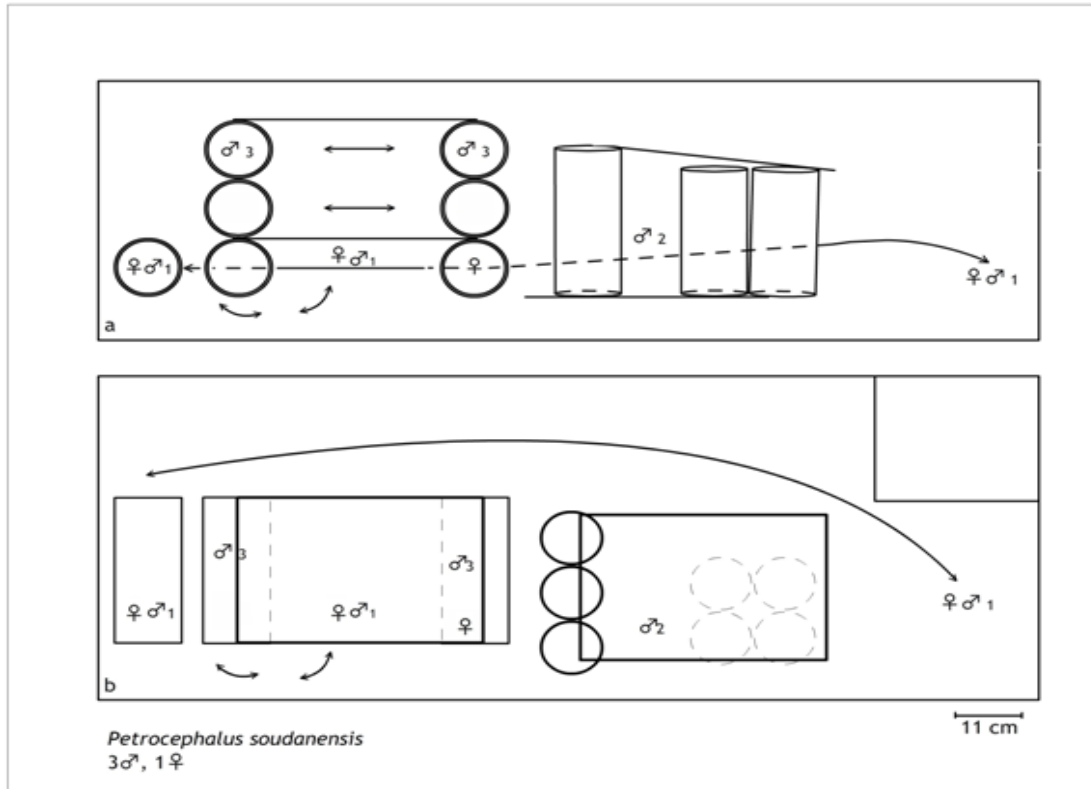


Fig. 16. Illustration of the swimming movements (arrows) during social and courtship behaviour of *Petrocephalus soudanensis* (1♀, 3♂); [a] Front view; [b] upper view.

After almost a year of experimenting, the breeding experiment was stopped since the female showed a thinner belly and spawning had not occurred. Some time after the onset of the imitation of the dry season (increase of conductivity) courtship behaviour also decreased. The swollen belly and the courtship behaviour indeed indicated successful gonad maturation. Spawning, however did not take place.

3.1.2 *Petrocephalus catostoma*

The first breeding experiment lasted 257 days comprising one female and four males. The course of the environmental factors is shown in **Fig. 17**. Conductivity was decreased from 875–155 $\mu\text{S}/\text{cm}$ with pH value varying from 8.4–7.8, whereas temperature fluctuated around 24.1–27.8 °C. Water level was kept constant at 40 cm. In the course of the breeding experiment one male and the female have established territories next to each other. Courtship behaviour as in *P. soudanensis* was not found.

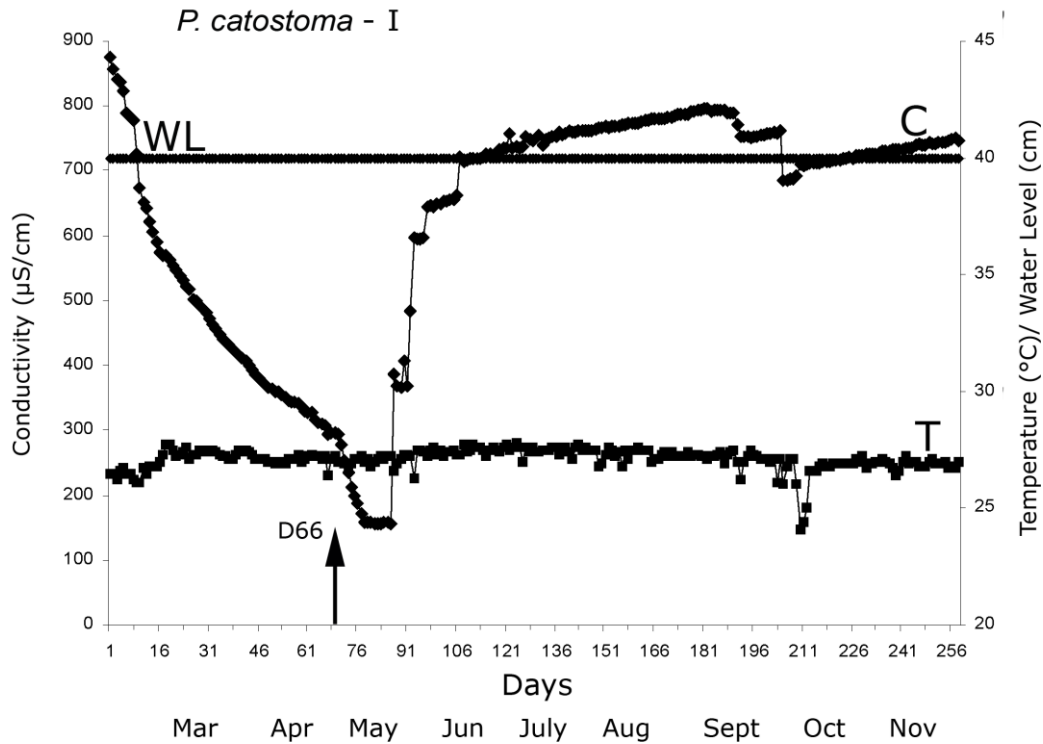


Fig. 17. Course of environmental factors, decreasing conductivity (C), constant water level (WL), and temperature (T) in the breeding experiment group I with a female and four males of *Petrocephalus catostoma* to provoke gonadal maturation and spawning. The female showed a swollen belly as first indication of gonad maturation on day 66. Neither courtship behaviour nor spawning occurred during the breeding experiment.

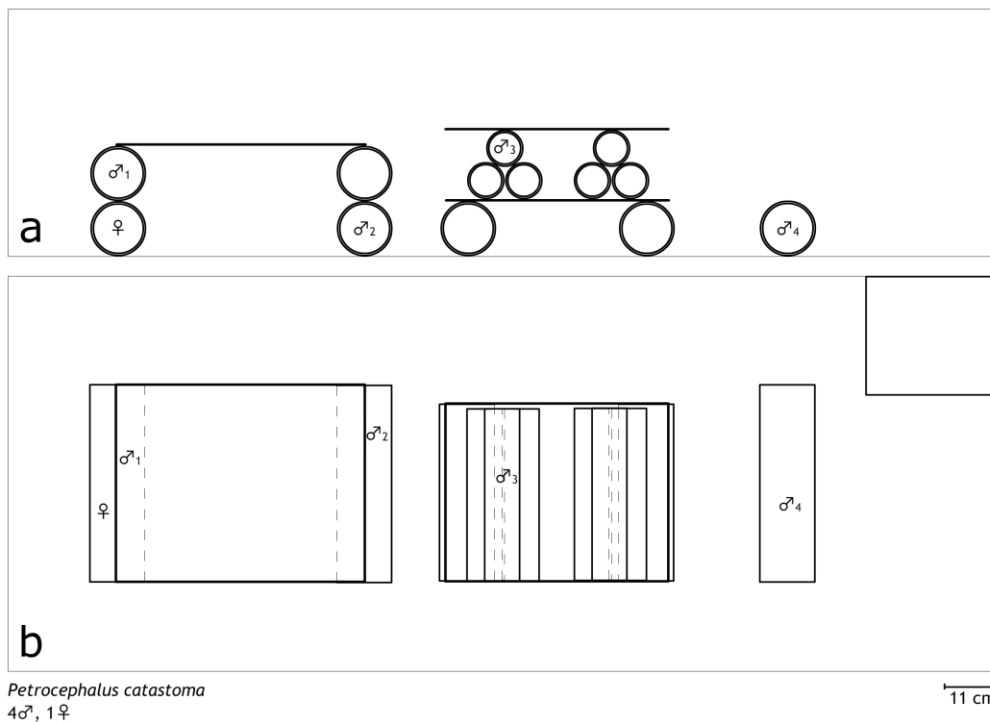


Fig. 18. Illustration of the position of the territories of 1 female and 4 males of *Petrocephalus catostoma* during the day time, [a] Front view; [b] upper view.

The female's belly did swell up during the 66 day experiment. The territories of the five fish are shown in **Fig. 18**. However, there was only a change in the position of the territories of 1 male and the female during the course of the breeding experiment. Within seven months, the specimens did show declining growth in terms of total length and total weight (see **Table 1**).

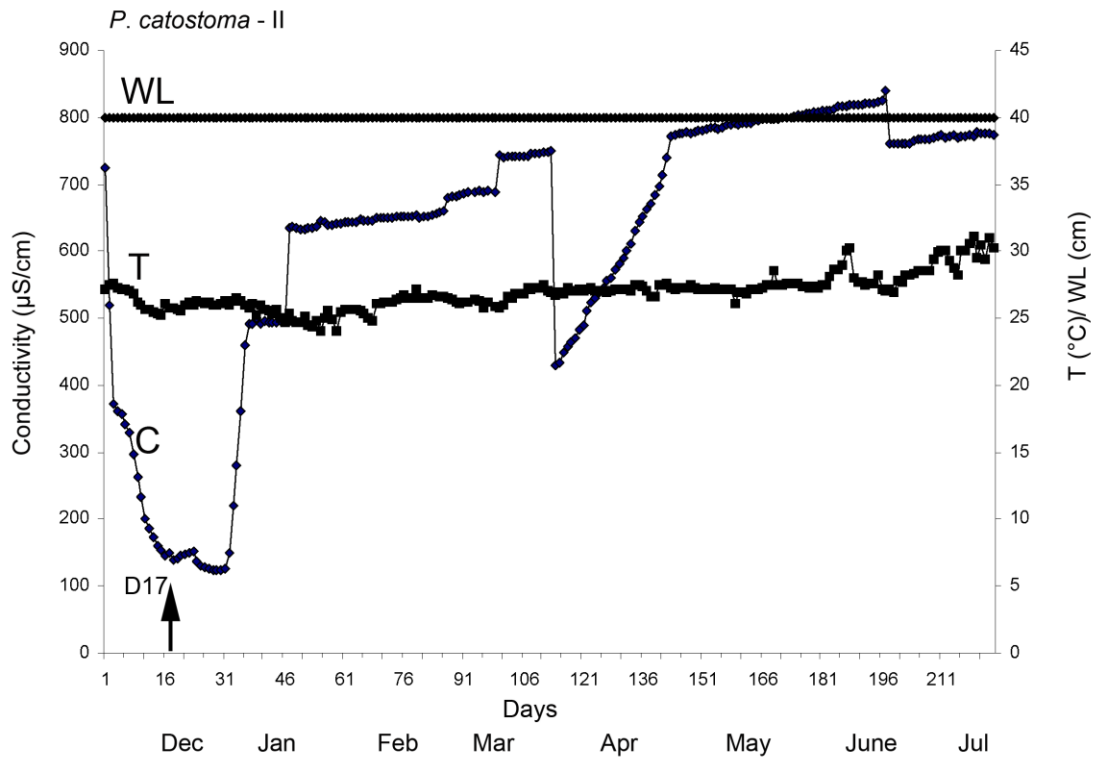


Fig. 19. Course of environmental factors, decreasing conductivity (C), constant water level (WL), and temperature (T) in the breeding experiment group II with a female and two males *Petrocephalus catostoma* to provoke gonadal maturation and spawning. Neither courtship behaviour nor spawning occurred during the breeding experiment.

Table 1. Measurement of total length and total weight of *Petrocephalus catostoma* during the first breeding experiment

20.03.2009				16.06.2009			12.10.2009		
No.	Sex	TW (g)	TL (cm)	Sex	TW (g)	TL (cm)	Sex	TW (g)	TL (cm)
1	♂1	20.13	11.60	♂1	18.72	11.60	♂1	16.29	11.60
2	♀	22.89	12.10	♀	21.43	12.10	♀	20.71	12.10
3	♂2	23.32	11.80	♂2	20.58	11.80	♂2	20.48	11.90
4	♂3	18.41	11.10	♂3	16.91	11.10	♂3	14.45	11.10
5	♂4	21.23	11.70	♂4	19.92	11.70	♂4	16.49	11.70

The second breeding experiment of *P. catostoma* lasted for 223 days involving the female and two males (**Fig. 19**), which were already used for the first breeding experiment (**Fig. 18**). The conductivity varied from 779 to 140 µS/cm, pH value from

8.3-6.8 and the temperature from 24.1–30 °C. Similar to the first breeding experiment, no unusual behaviour was observed throughout the entire period. Indication for the female's swollen belly was observed here on day 17, way earlier than in the first breeding experiment. However, neither courtship behaviour nor spawning was observed afterwards.

3.2 Family Pantodontidae, *Pantodon buchholzi*

Figure 20 and **21** present the course of applied environmental factors; water level (WL), conductivity (C) and temperature (T) in breeding group I (7♂, 5♀) during period of 328 days and in breeding group II (1♂, 2♀) within 221 days of breeding experiment, respectively. Two fish (1♂, 1♀) of the first group showed signs of courtship behaviour during the decreasing conductivity. However, intense courtship lasted only for several days starting from day 54 and lasting until day 67. This pair was often seen swimming close to each other and the male swam actively to attract the female's attention. This observation took place mostly during daytime.

The two experimental tanks had been equipped differently, which provoked two different types of social behaviours. In the first group of *P. buchholzi*, the tank was furnished with black plastic shreds and several plastic tubes. The fish adapted very well to this environment, hiding between the plastic shreds. During feeding, the fish reacted differently compared to the second group, whose tank had not been supplied with decoration. These fish calmly waited for the crickets, which were running on the water surface, to approach them. On the other hand, the fish of the second group swam actively and very quickly to catch the crickets on the water surface.

In addition, three breeding experiments with combination (C) of I (1♀, 1♂), II (5♀, 4♂), and III (4♀, 5♂) were performed in three different tanks of 90 x 60 x 53 cm for a time period of two months with several trials of drastically decreasing conductivity. However, neither a swollen belly nor courtship behavior was observed during these trials. Six (2♀, 4♂) specimens were kept in the tank for nearly eight months, whereas the rest of specimens were put to death and dissected (see **Table 2**). The six specimens were measured monthly in total length and total weight. The fish often had been seen swimming on the surface and somehow lost their appetite towards the crickets. However, neither courtship nor spawning took place. Four specimens died one after another due to some unidentified illnesses.

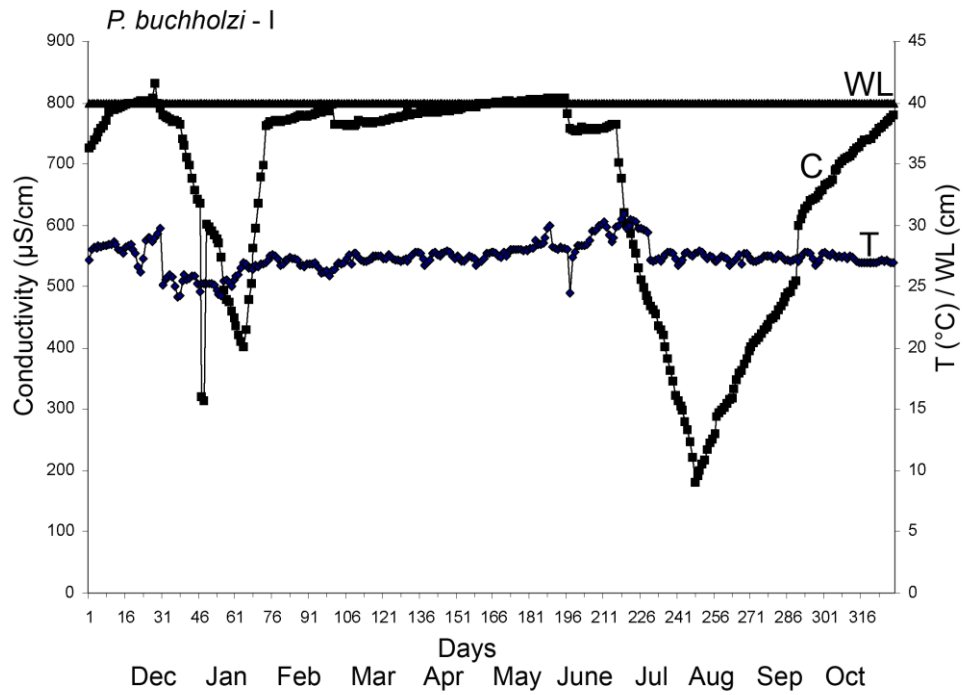


Fig. 20. Course of environmental factors, decreasing conductivity (C), constant water level (WL), and temperature (T) in the breeding experiment group I (7♂, 5♀) of *Pantodon buchholzi* to provoke gonadal maturation and spawning. No spawning occurred during the breeding experiment.

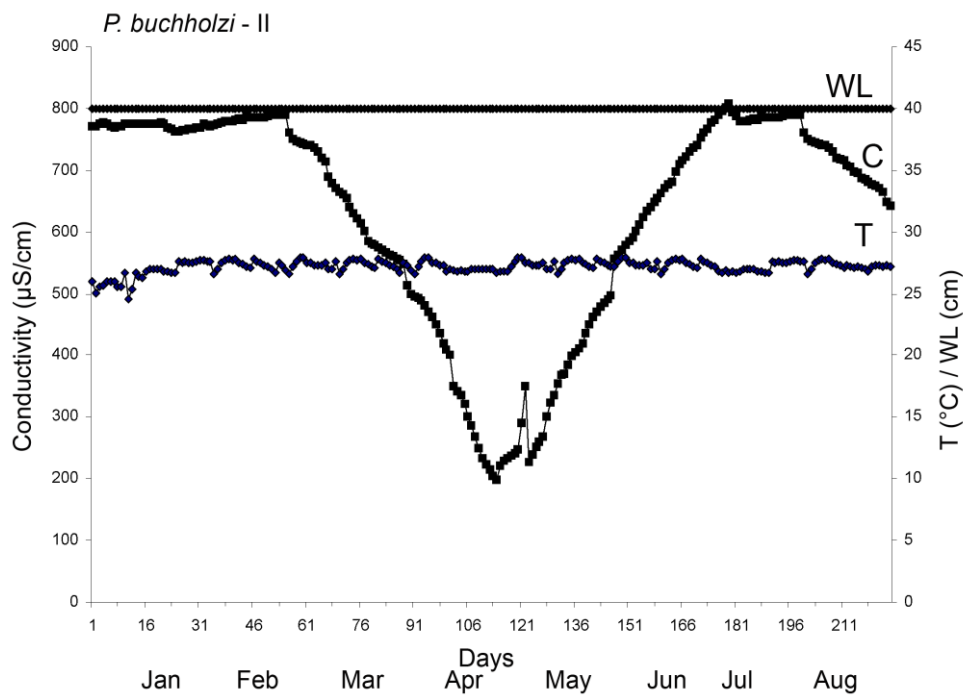


Fig. 21. Course of environmental factors, decreasing conductivity (C), constant water level (WL), and temperature (T) in the breeding experiment group II of *Pantodon buchholzi* with two females and a male, to provoke gonadal maturation and spawning. No spawning observed.

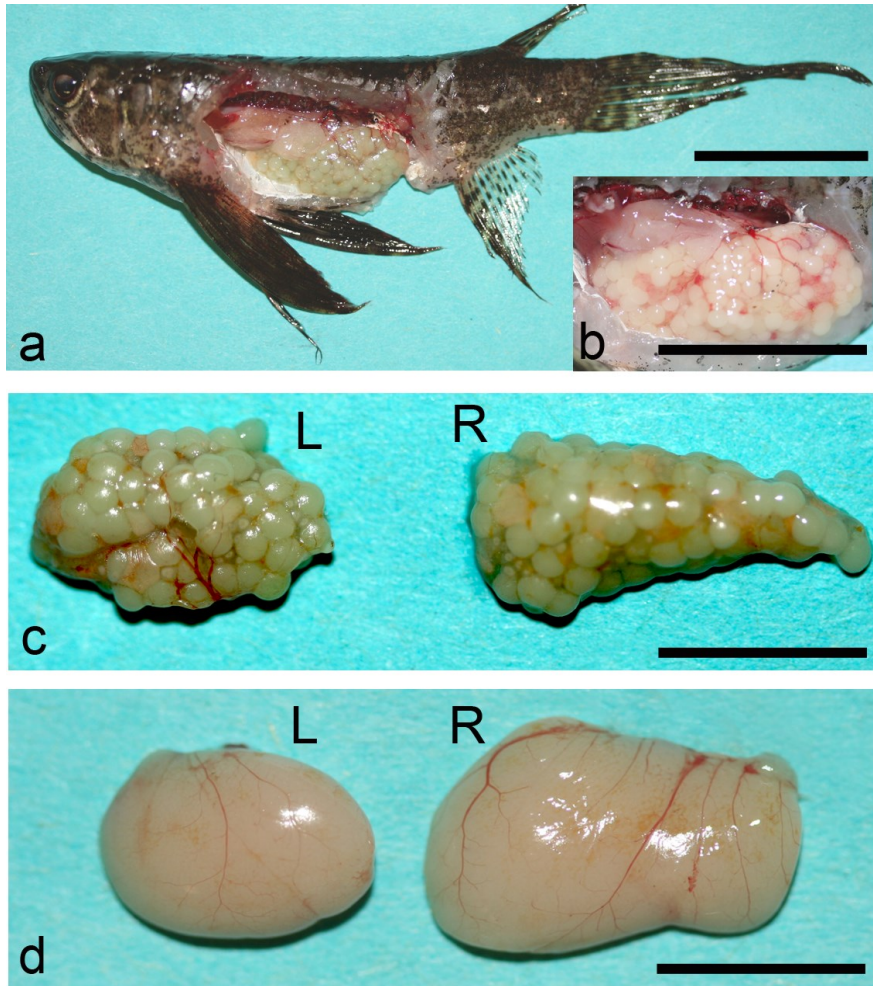


Fig. 22. *In situ* condition of the gonads in *Pantodon buchholzi*. [a] Light greenish female gonads surrounded with fat tissue, total length 10.3cm whereas [b] some gonads were found of whitish color. [c] Gonads taken out left (0.22 gr) and right (0.21 gr). [d] Testes freshly taken out, left (0.23 gr) and right (0.30 gr). Scale bar = 20 mm (a), 10 mm (b, c, d).

In situ situation in all specimens of *Pantodon buchholzi* demonstrated two different colours of the female's gonad - greenish and whitish (**Fig. 22**). The ovary is made up of left and right parts. The total gonad weight of the female (TGW) ranges between 0.08-0.73 g (left) and between 0.1–0.95 g (right), whereas the male's total gonad weight (TGW) varies between 0.02–0.23 g (left) and 0.02–0.3 g (right). **Table 2** below presents the percentage of the maturity coefficient (MC) of 41 dissected specimens including total length (TL), total weight (TW) and total gonad weight (TGW) of *Pantodon buchholzi*.

Table 2. Maturity coefficient (MC) of dissected *Pantodon buchholzi* at the end of the experimental period, with total length (TL), total weight (TW), and total gonad weight (TGW) data from all groups (I, II, CI-Combination I, CII-Combination II, CIII-Combination III, R-Rest)

No	Sex	TL (cm)	TW (cm)	TGW (g)		MC (%)		Group
				L	R	L	R	
1	♂	10.00	6.62	0.07	0.09	1.10	1.38	I
2	♂	9.90	7.41	0.09	0.12	1.23	1.64	I
3	♂	9.80	6.42	0.06	0.11	0.94	1.70	I
4	♂	10.00	6.81	0.09	0.08	1.30	1.18	I
5	♂	10.00	7.53	0.05	0.10	0.67	1.30	I
6	♂	9.70	6.54	0.08	0.11	1.30	1.70	I
7	♂	9.10	5.49	0.15	0.12	2.80	2.23	I
8	♀	9.30	6.71	0.43	0.53	6.80	8.60	I
9	♀	9.00	6.01	0.31	0.36	5.40	6.37	I
10	♀	9.50	5.40	0.13	0.09	2.70	1.70	I
11	♀	9.30	5.43	0.09	0.10	1.70	1.90	I
12	♀	9.00	5.80	0.06	0.10	1.05	1.75	I
13	♂	9.30	6.55	0.08	0.10	1.20	1.70	II
14	♀	9.30	4.58	0.13	0.19	2.90	4.32	II
15	♀	9.00	5.80	0.12	0.13	2.10	0.20	II
16	♂	10.00	5.79	0.02	0.02	1.34	1.34	C I
17	♀	8.00	3.96	0.08	0.11	2.06	2.85	C I
18	♂	10.00	7.35	0.08	0.11	1.20	1.50	C II
19	♂	8.80	5.62	0.09	0.10	1.60	1.26	C II
20	♂	9.30	6.24	0.09	0.07	1.50	1.60	C II
21	♂	9.60	6.60	0.14	0.18	2.16	2.80	C II
22	♀	8.50	4.22	0.21	0.27	5.23	6.80	C II
23	♀	8.70	5.37	0.25	0.27	4.80	5.30	C II
24	♀	8.50	6.07	0.42	0.55	7.40	9.90	C II
25	♀	10.30	7.68	0.22	0.21	2.90	2.80	C II
26	♀	10.00	8.90	0.73	0.95	0.80	1.20	C II
27	♂	9.50	6.18	0.07	0.11	1.13	1.79	C III
28	♂	8.00	4.42	0.05	0.05	1.14	1.14	C III
29	♂	9.40	6.34	0.08	0.09	1.27	1.40	C III
30	♂	9.00	4.34	0.13	0.14	3.30	3.33	C III
31	♂	9.00	4.40	0.10	0.10	2.30	2.30	C III
32	♀	9.30	6.00	0.11	0.10	1.80	2.14	C III
33	♀	9.60	7.76	0.60	0.70	7.80	9.10	C III
34	♀	9.80	7.48	0.29	0.50	4.00	7.10	C III
35	♀	11.00	9.36	0.22	0.38	2.40	4.20	C III
36	♀	9.80	6.64	0.31	0.40	4.90	6.40	R
37	♀	9.50	6.30	0.20	0.30	3.30	5.00	R
38	♂	10.30	7.68	0.22	0.21	2.90	2.80	R
39	♂	10.30	7.93	0.11	0.16	1.40	2.06	R
40	♂	10.50	9.77	0.23	0.30	2.40	3.16	R
41	♂	10.90	9.78	0.18	0.27	1.87	2.83	R

3.3 Family Notopteridae

3.3.1 *Xenomystus nigri*

The first breeding experiment lasted for 470 days. Decreasing conductivity took place for three times within the first period (**Fig. 23**) ranging from 901 to 301 $\mu\text{S}/\text{cm}$, with temperature varying from 24.3–29.9 °C and pH value ranging from 8.4–6.6. Water level was always kept constant at 40 cm.

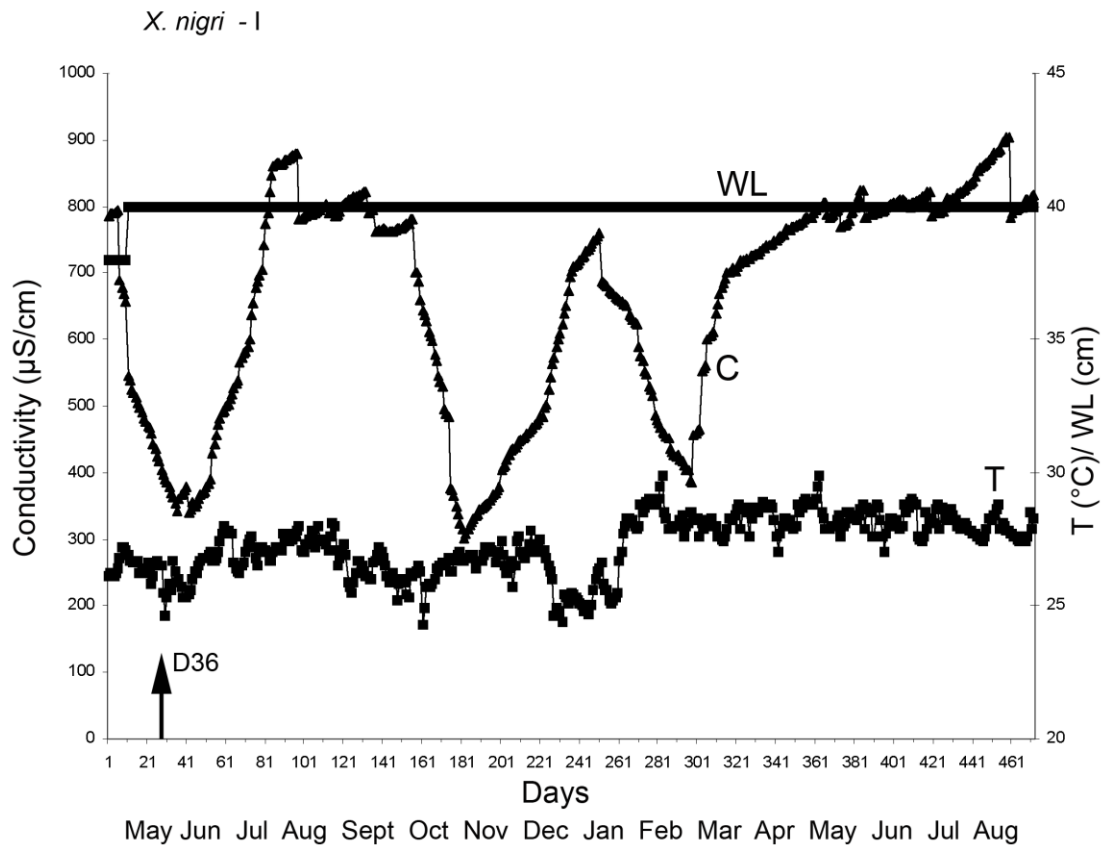


Fig. 23. Course of environmental factors, decreasing conductivity (C), constant water level (WL), and temperature (T) in the breeding experiment group I for a 16-month period of *Xenomystus nigri* (9♀, 7♂), to provoke gonadal maturation and spawning. First swollen belly was seen in two females on day 36, arrow.

First indications for the swelling bellies of three females was seen on day 36 at 342 $\mu\text{S}/\text{cm}$. Courtship behavior took place for a few times during the experiment, however, spawning was never observed although some females and males continued showing obviously swollen bellies. Three females died throughout the course of the experiment.

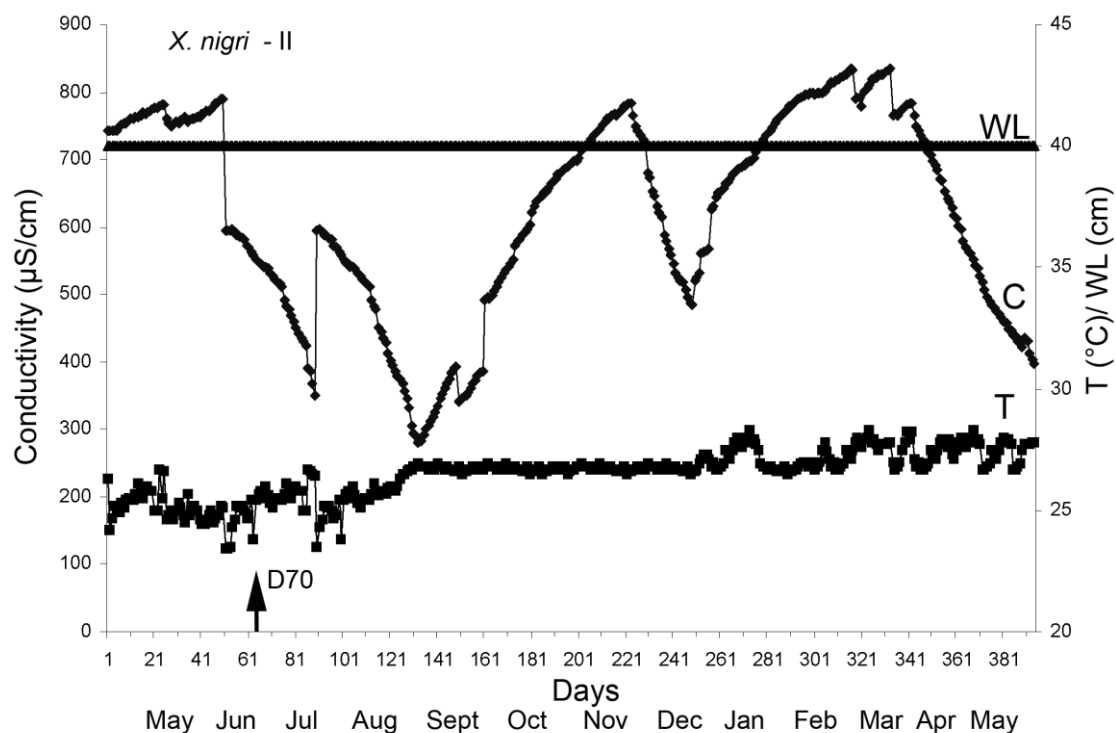


Fig. 24. Course of environmental factors, decreasing conductivity (C), constant water level (WL), and temperature (T) in the breeding experiment group II for 13 months period of *Xenomystus nigri* (9♀, 1♂), to provoke gonadal maturation and spawning. First three females with swollen bellies were seen on day 70, arrow.

The three selected pairs, showing significantly swollen bellies, were subjected to the application of hormonal injection. They were pair I (♂1: TW= 26.7 g; TL= 16.5 cm and ♀1: TW= 29.14 g; TL= 17 cm), pair II (♂2: TW= 21.58 g; TL= 15.5 cm and ♀2: TW= 29.14 g; TL= 17 cm), and pair III (♂3: TW= 21.52 g; TL= 15.5 cm and ♀3: TW= 24.67 g; TL= 16.9 cm). Five hormonal injections were given to these three pairs on day 389, 395, 402, 408, and day 415. Apparently those five hormonal injections seemed to have no particular impact on inducing spawning, although intense courtship behaviours were often observed within these three pairs. The pairs indeed were seen swimming very closely to each other during day time. In fact, double of the concentration of the regular hormonal injection was given on the last day (day 415) of the trial to all of the selected specimens, however still nothing happened. Nevertheless, triggered by decreasing conductivity, gonad maturation could successfully be induced on this species, which was apparent by its significant changes around the bellies.

The second breeding experiment lasts for 394 days consisting nine females and a male. The first swollen belly was observed at day 70 in three females. Similar to the breeding experiment 1, no spawning occurred. Two females died throughout the course of the experiment.

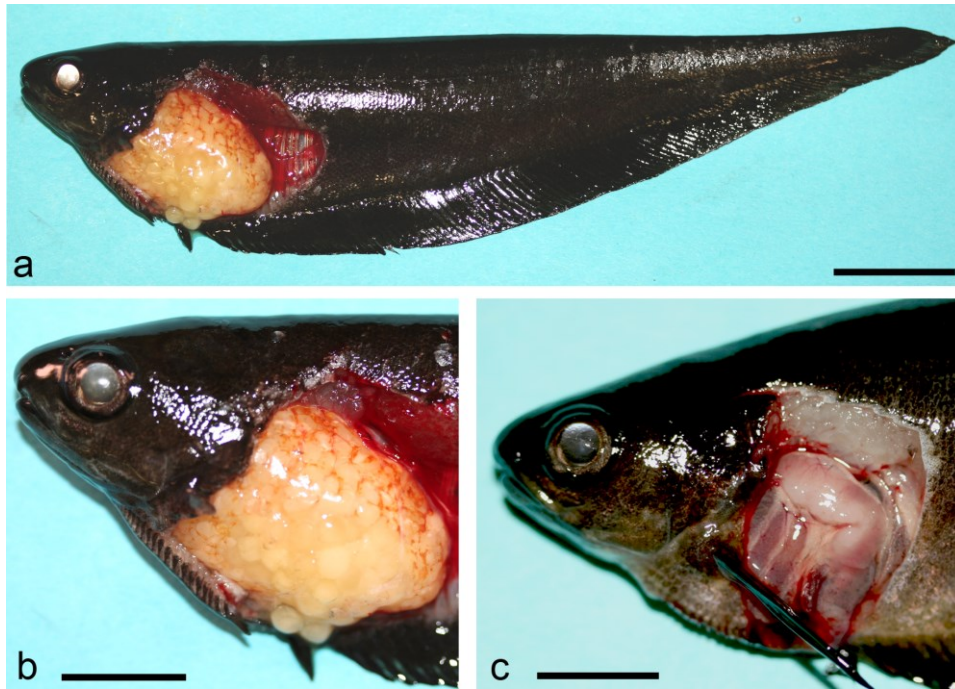


Fig. 25. *In situ* condition in *Xenomystus nigri*. [a] Female's gonad occupies almost 80 % of internal organ, total length 16 cm and total weight of 22.38 g. [b] Gonad consists of different stadium of oocytes, larger magnification. [c] Male's *in situ* with undeveloped testes, total length 15.4 cm and total weight of 17.27 g. Scale bar = 20 mm (a), 10 mm (b, c, d).

Figure 25 shows the *in situ* condition of *Xenomystus nigri* presenting the ovary (**Fig. 25a**) and testis (**Fig. 25b**). *X. nigri* possesses only a single ovary positioned on the left side of the abdominal cavity. The ovary consisted of different stadia of oocytes. A single testis was found laterally in a similar position in the male's abdomen, lying on the apex of the coiled digestive tract. Maturity coefficient value of figured female is 7.7 % and the male has 0.23 %. **Table 3** presents the percentage of the maturity coefficient (MC), including total length (TL), total weight (TW) and total gonad weight (TGW), of 21 dissected specimens of *Xenomystus nigri* at the end of all experiments.

Table 3. Maturity coefficient (MC) of dissected *Xenomystus nigri* at the end of experimental period, with total length (TL), total weight (TW), and total gonad weight (TGW) data from breeding group I and II

No	Sex	TL (cm)	TW (g)	TGW (g)	MC (%)	Breeding Group
1	♀	14.30	15.80	0.94	6.50	I
2	♀	13.90	14.31	0.44	3.20	I
3	♀	15.70	22.00	1.56	7.63	I
4	♀	12.80	10.60	0.50	4.95	I
5	♀	16.30	22.38	1.60	7.70	I
6	♀	17.50	28.28	1.06	3.89	I
7	♂	14.90	17.78	0.20	1.10	I
8	♂	14.00	14.61	0.03	0.20	I
9	♂	13.70	13.60	0.02	0.15	I
10	♂	14.60	16.54	0.10	0.30	I
11	♂	17.00	27.24	0.10	0.40	I
12	♂	15.50	8.62	0.10	0.80	I
13	♂	15.50	17.27	0.04	0.20	I
14	♀	16.40	21.42	0.11	0.50	II
15	♀	15.40	18.45	0.11	0.60	II
16	♀	14.80	15.58	0.06	0.40	II
17	♀	15.30	22.05	1.70	8.30	II
18	♀	17.80	19.25	0.68	3.66	II
19	♀	16.90	24.11	0.66	2.80	II
20	♀	14.60	15.82	0.78	5.18	II
21	♂	15.70	21.84	0.30	1.40	II

3.3.2 *Notopterus notopterus*

3.3.2.1 Reproduction

In *N. notopterus* only the left gonad is developed (dissection of 16 specimens (9♀, 7♂)). The first breeding experiment was performed with one male and two females (**Fig. 26**). The variation of conductivity was fulfilled, based on experiments with mormyrids and gymnotiforms (Kirschbaum and Schugardt, 2004), which had shown that decreasing conductivity can elicit gonad maturation. A swollen belly as a sign for gonad maturation was seen for the first time in female No. 1, 33 days after the onset of the experiment.

For observational purposes, a tiny part of the caudal fin of this female was cut out to easily identify the specimen. First time spawning occurred on day 198 of the experimental period, followed by 19 spawning events with irregular intervals (see **Table 4**) within a five-month period. Female No. 2 spent most of the time alone on the other part of the tank without interrupting the pair during courtship and spawning behaviour.

The three fish were 269 mm (♀ No.1), 236 mm (♀ No. 2), and 250 mm (♂) in total length (TL) and varied in total weight of 99.25 g, 83 g and 118 g, respectively. On the

following day the conductivity was decreased from 780–620 $\mu\text{S}/\text{cm}$ (**Fig. 14**) to start the experiment.

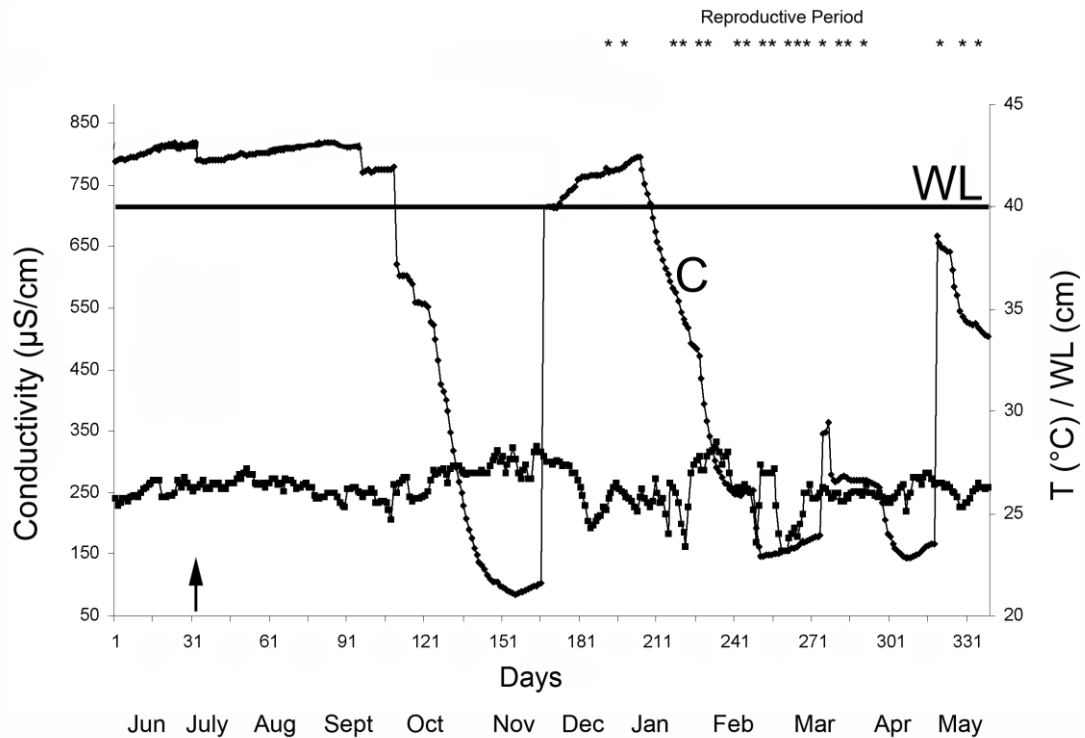


Fig. 26. Course of environmental factors conductivity (C), water level (WL), and temperature (T) during 339 days in breeding tank I containing two females and one male of *Notopterus notopterus*. 20 spawning occurrences of the female No. 1 were observed within a 5-month period. The breeding experiment was commenced after a 3 months acclimatization period. Note the first swollen belly of the female No. 1 (Arrow). * refers to observed spawning.

The second breeding experiment comprised one male (TL= 261 mm, TW = 110 g) and female (TL= 236, TW= 83 g) (**Fig. 27**). A swollen belly of the female concurrently with courtship behaviour was first observed on day 62. However, no spawning occurred during this initial courtship period. Around 15 times, intense courtship behaviour was observed mostly from 12.00–6.00 pm throughout a five-month period. However, spawning occurred only six times. The first spawning of this pair was found on day 178, around 5.30–7.00 pm, whereas the following spawning mostly took place from 8.00 to 2.30 pm. Maturity coefficient (MC) values of specimens used in breeding experiment I at the end of the experimental period are 8.46 % (♀1), 8.75 (♀2) and 1.12 (♂), corresponding to **Figure 26**. Whereas in breeding experiment II (**Figure 27**) the maturity coefficient (MC) for the single male and female are 0.76 % and 7.52 %, respectively.

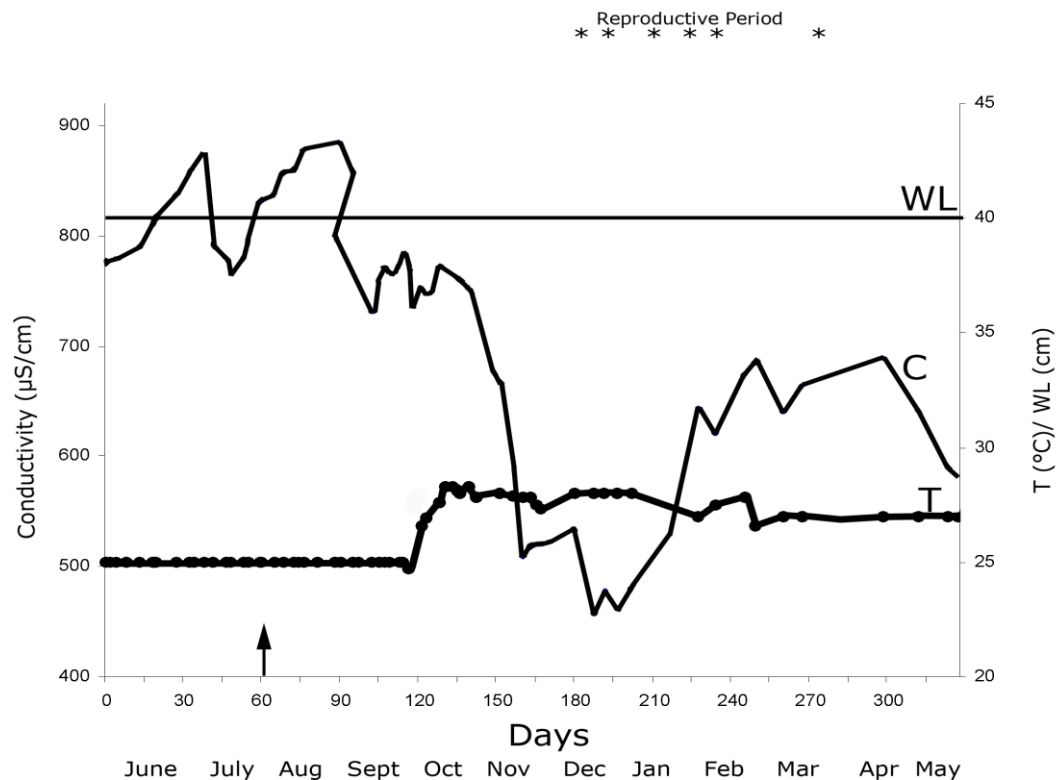


Fig. 27. Course of environmental factors during 331 days, conductivity (C), water level (WL) and temperature (T) in breeding tank II containing one female and one male *Notopterus notopterus*. An irregular spawning interval was observed for 6 times within a 6-month experimental period. The breeding experiment was started after 40 days of acclimatization. Note the first swollen belly of the female (Arrow). * refers to observed spawning.

Courtship behaviour was regularly observed throughout the entire daytime. Overall there were five steps of courtship behaviour observed during these breeding experiments: 1) The male followed or swam alongside the female as the female often swam faster than the male, 2) Male touched female's belly with his mouth several times while the female actively swam up and down in front of the male, 3) Male quivered the female by swinging rapidly its tail against the side of the female's body, to which the female reacted by swimming quickly to the other side of the tank and then returning directly to its previous position, 4) A male and a female stayed still in a corner of the tank until the male suddenly swam to the other side on its own, 5) The male later on was actively approached by the female and led the female to the spawning site. Two representative pictures of courtship behaviour from the second breeding experiment are presented in **Figure 28**. In the particular case of the second breeding pair, mostly during this intense courtship behaviour the male was actively seen preparing a suitable spawning site, by removing gravels and cleaning the spawning site with its mouth (**Fig. 29**).

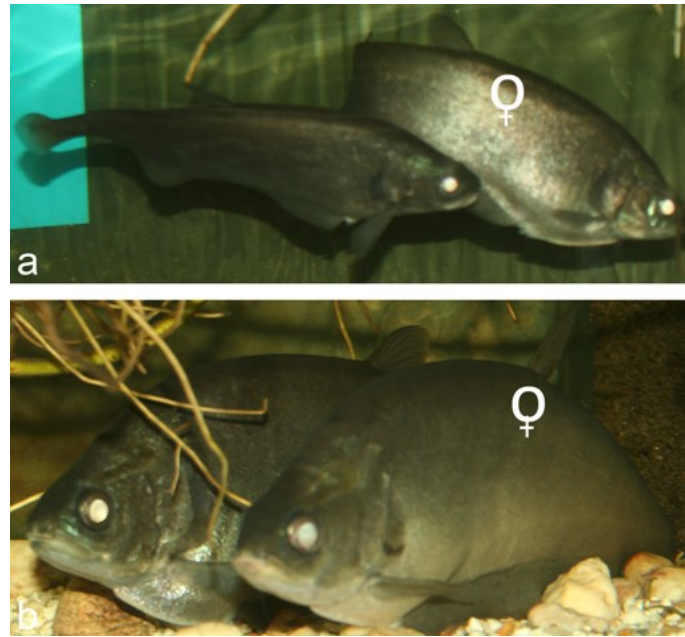


Fig. 28. Two elements of courtship behaviour in *Notopterus notopterus*. [a] Around the spawning site the male touched the female's abdomen with its mouth and [b] the pair remained together on the right side of the tank.

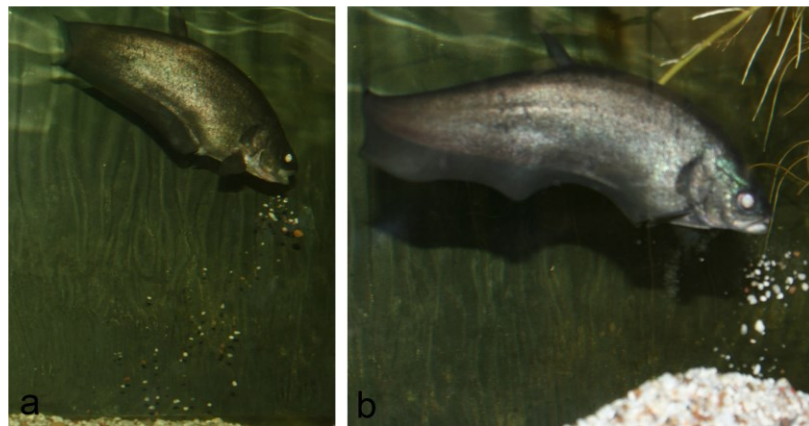


Fig. 29. Intense removing gravels activity was found in breeding experiment II. Male was actively seen collecting gravel the whole day and removed it to somewhere else nearby. These activities [a, b] will stop, once the preferred ground is completely gravel-free.

The 20 successful spawning events of the first breeding experiment were observed during day time. The pair seemed ready to spawn as the female showed a swollen belly and the male was apparently attracted by the female. Soon after the female had laid some eggs on the preferred substrate, the male quickly fertilized the eggs while the female was still nearby. When spawning was finished, the male remained at the spawning site, while the female left for the other side of the tank. The genital papilla of the female appears bigger than usual during courtship and spawning. It is approximately 5–7 mm in length.

The complete sequence of the spawning number two (11.01.2010) from the first breeding pair is illustrated in six elements as shown in **Figure 30**: a) Male pushed the female with his mouth to the spawning substrate, b) after the male fertilized the eggs, the pair was seen together very close to their newly spawned eggs, c) the female laid some eggs for the second time, which were then directly fertilized by the male, d) male pushed the female with his head to lay some eggs on the other side of the rock, the female seemed to be in a leaning position of 30° angle, e) after the female laid some eggs, the male directly fertilized the eggs, while the female still stayed very close, somehow touching the male's belly with its head, f) the female laid eggs for the last time in this sequence.

Table 4. Overview of spawning (Sp) events in breeding group I (2♀, 1♂) and breeding group II (1♀, 1♂) with number of eggs, pH value and temperature (T) per individual spawning

Breeding Group I					Breeding Group II				
Sp. Nr.	Sp. Date	Nr. Eggs	pH	T (°C)	Sp. Nr.	Sp. Date	Nr. Eggs	pH	T (°C)
1	04.01.2010	50	8.5	26	1	16.12.2009	87	6.5	28
2	11.01.2010	68	8.2	26.2	2	23.12.2009	75	7.0	28
3	19.01.2010	90	7.9	25.7	3	14.01.2010	50	8.2	27.8
4	21.01.2010	50	7.7	25.3	4	17.02.2010	47	7.8	27.8
5	02.02.2010	50	7.7	27.8	5	28.02.2010	45	7.6	27
6	03.02.2010	25	7.7	27.1	6	27.03.2010	51	6.0	27
7	16.02.2010	74	7.5	26.2			355		
8	17.02.2010	30	7.5	26.3					
9	25.02.2010	105	7.0	25.9					
10	02.03.2010	225	7.0	27					
11	12.03.2010	113	6.5	24.9					
12	17.03.2010	27	6.5	26.4					
13	23.03.2010	120	6.7	26.2					
14	28.03.2010	40	7.3	26					
15	29.03.2010	180	7.3	25.6					
16	04.04.2010	70	7.6	26.1					
17	13.04.2010	15	7.9	26					
18	10.05.2010	118	8.3	26.3					
19	17.05.2010	30	8.0	25.7					
20	25.05.2010	80	7.7	26.3					
		1560							

Regarding most of the spawning events of the first breeding pair, nearly all the eggs were spawned at the same substrate and location. The eggs were attached to the bottom side of larger stones. Nevertheless, in the last two spawning events of this breeding pair, eggs were found outside of the common area: on the edge of the filter (**Fig. 31c**), even though some larger stones were positioned nearby. The female deposited circa 15–225 eggs per spawning and 1560 eggs during the 20 spawning occurrences (see **Table 4**).

Meanwhile, the second breeding pair always spawned underneath or nearby large stones. Within six spawning events, the female deposited around 355 eggs in total. Detail numbers of each spawning is presented in **Table 4**.

Parental care is performed by the male. This implies defending the nest against other specimen in the tank and guarding the eggs (**Fig. 32**). For two days after spawning the male feeds on significantly less food and protects the eggs aggressively, also against the female.

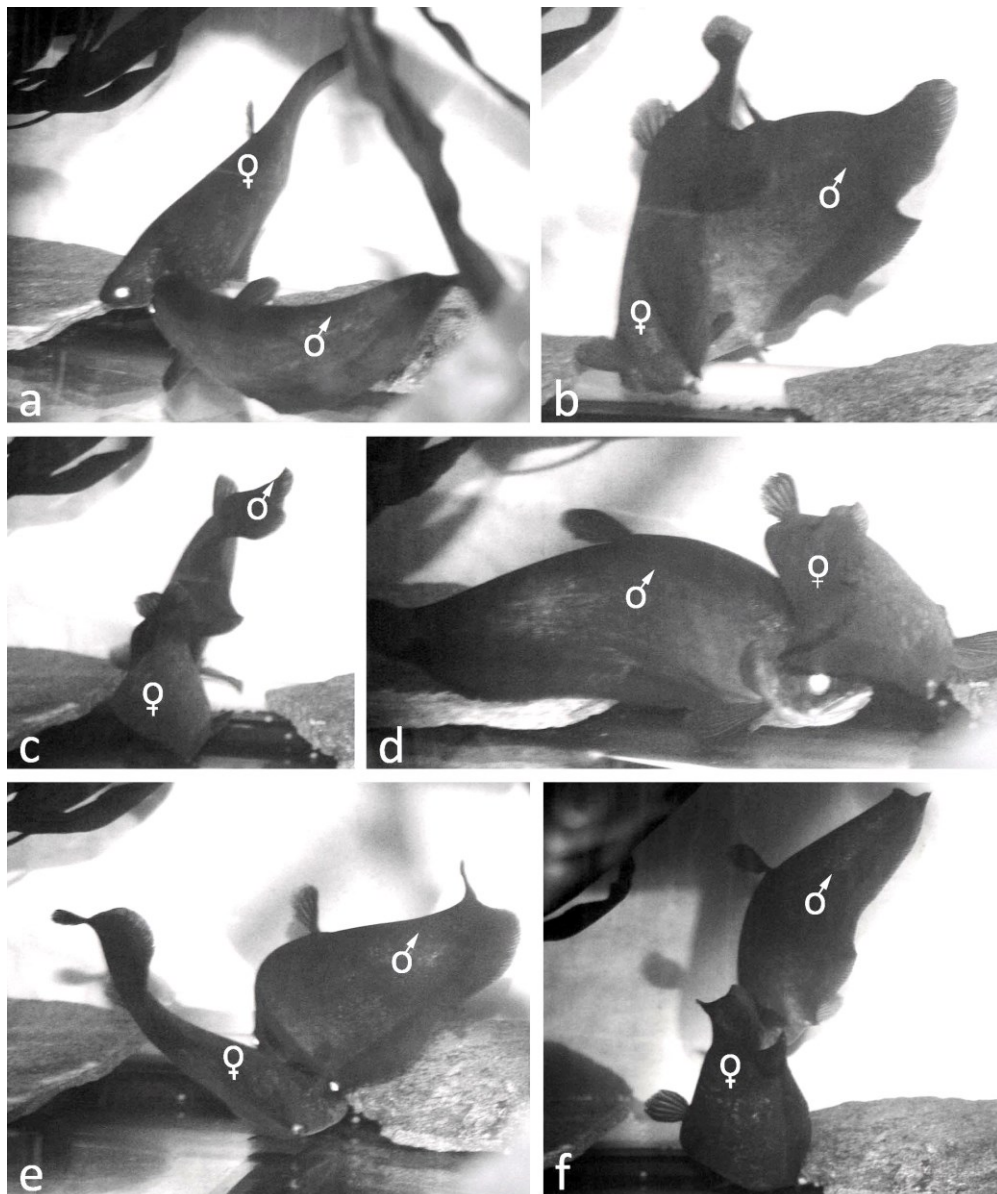


Fig. 30. Spawning sequence of *Notopterus notopterus*. Egg deposition occurred in different positions, while the male accompanied the female in every action. [a] The male gently pushed the female on its belly with its mouth, to lead her to the desired spawning substrate. [b] Both were seen staying very closely together after the male fertilized the eggs. [c] The second spawning of the female, followed by the fertilization of the male. [d] Male pushes the female again to other substrate nearby the first location of spawning. [e] The male directly fertilized the eggs. [f] The female spawns the eggs for the last time on this occasion.

3.3.2.2 *In situ* condition of mature and immature (F1) specimens

Figure 33 is the *in situ* condition of a dissected female *Notopterus notopterus* presenting an immature gonad (**Fig. 33a**) and a mature gonad (**Fig. 33b**), respectively. *N. notopterus* possesses only one single ovary positioned laterally in the abdominal cavity on the coiled intestine on the left side of the fish. The immature gonad contains mostly oocytes in stadium 1 with their very dominant whitish colour as seen in **Figure 33a**. The picture of the immature gonad was taken from a female of 12 cm in total length and total weight of 10.32 g.

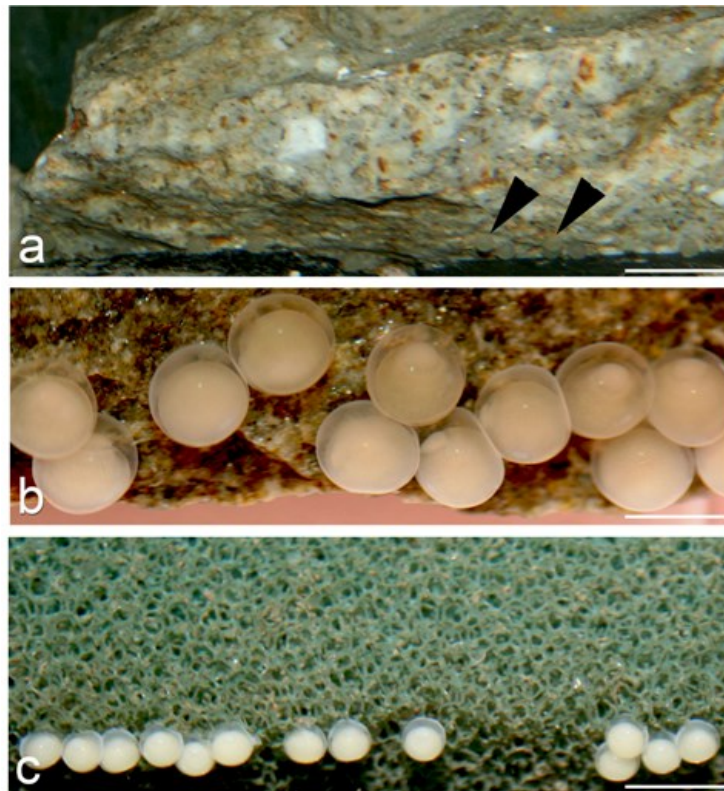


Fig. 31. Preferred spawning sites of *Notopterus notopterus*. [a] The eggs attached to the underside (double arrowhead) [b] on the edge of a large stone. [c] Eggs were also found on the edge of the filter. Scale bar = 20 mm (a); 4 mm (b); 10 mm (c).

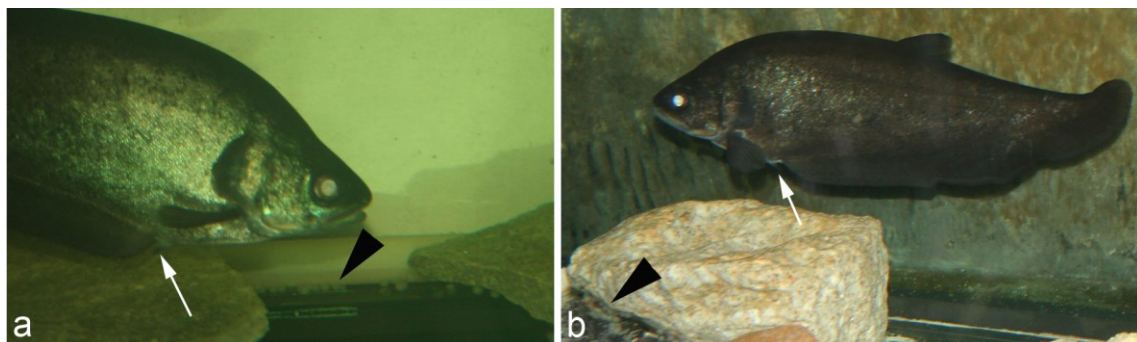


Fig. 32. Parental care of *Notopterus notopterus* performed by the male. [a] Male in breeding tank 1 and [b] Male in breeding tank 2. Both males were always seen nearby the spawning substrates, note the male's genital papilla (white arrow) and newly spawned eggs (black arrowhead).

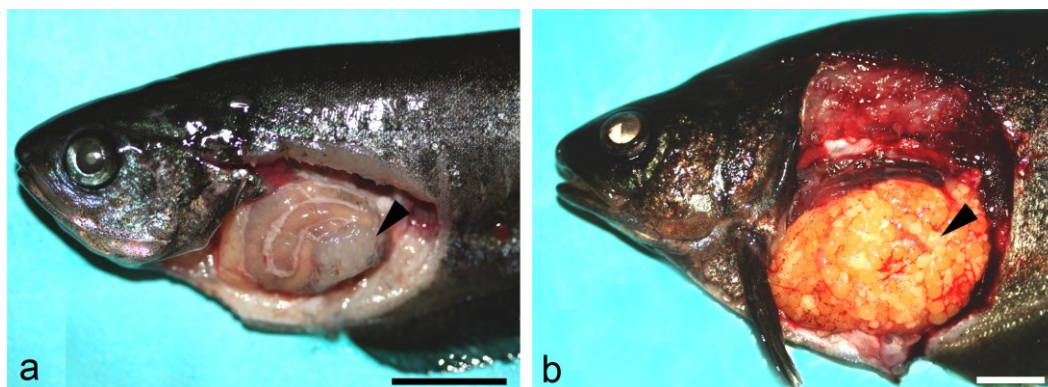


Fig. 33. *In situ* condition in female *Notopterus notopterus*. [a] An immature gonad, containing only oocytes in stadium 1, total length (TL) 12 cm. [b] Mature gonad consists of different stadia of oocytes, TL= 23 cm. Black arrowhead points the gonads. Scale bar = 20 mm (a), 10 mm (b, c, d).

The mature gonad of a female of 23 cm total length and 111.5 g weight was yellowish-orange, occupied almost all of the space in the abdominal cavity, showed oocytes in different stages and was surrounded with fat tissue. Maturity coefficient values for the two females were 0.68 % and 8.46 %, respectively. **Table 5** presents the percentage of the maturity coefficient (MC) in 11 dissected specimens of *Notopterus notopterus*.

Table 5. Maturity coefficient (%) of dissected *Notopterus notopterus*, as relating to total length (TL), total weight (TW), and total gonad weight (TGW) data

No	Sex	TL (cm)	TW (g)	TGW (g)	MC (%)
1	♂	23.70	129.94	0.45	0.34
2	♂	23.70	115.44	0.77	0.67
3	♂	27.20	170.40	1.26	0.74
4	♂	26.00	156.84	0.83	0.53
5	♂	25.50	161.64	1.18	0.73
6	♀	24.20	124.37	5.68	4.80
7	♀	25.20	131.47	7.44	5.90
8	♀	24.50	133.80	6.54	5.13
9	♀	21.60	82.15	2.76	3.47
10	♀	22.80	108.39	5.63	5.47
11	♀	22.10	112.40	7.64	7.29

3.3.2.3 External features of the egg

Fertilized eggs (**Fig. 34**) are adhesive, yellowish, and spherical with 3.8–4 mm in diameter. The egg envelope has many external ridges which are centred around the micropyle (**Fig. 35**).

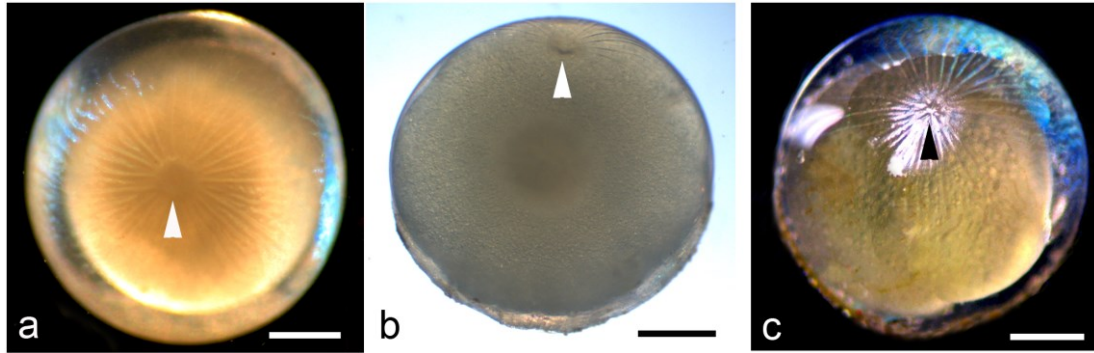


Fig. 34. Newly spawned eggs of *Notopterus notopterus*. [a] The egg envelope has many spiralling ridges originating from the micropyle. [b] The micropyle (m), located at the animal pole, is clearly marked as an opening in the egg envelope of the ovulated egg. [c] View of an egg close to the animal pole. Arrowhead points to the micropyle. Scale bar = 1 mm.

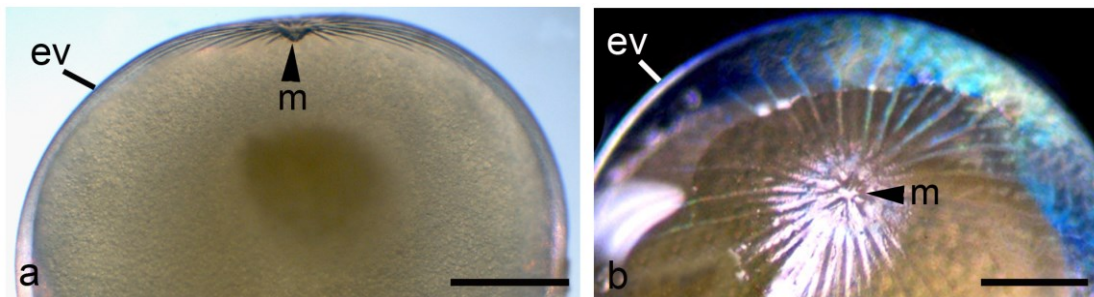


Fig. 35. Micropyle of *Notopterus notopterus*. [a] Larger magnification of **Fig. 34b**, note the depth of the micropyle (m) (arrowhead). [b] Larger magnification of **Fig. 34c**, showing the spiralling ridges seen from above. ev= egg envelope. Scale bar = 1 mm.

3.3.2.4 Development

Eggs were incubated at 27 °C. The terminology of the development of *Notopterus notopterus* follows the classifications of Balon (1975). There are five periods: the embryonic period (I), the larval period (II), the juvenile period (III), the adult period (IV) and the senescent period (V). The last period will not be discussed in this work due to the lack of information on this period in *N. notopterus*. Time of spawning is used as the time zero in age determination; it is presented as hrs:min or days.

3.3.2.4.1 The embryonic period

3.3.2.4.1.1 The cleavage phase

Stage 1: zygote-one cell

At 1:10, a discrete and distinct brownish pattern emerged to the top of the egg, the blastodisc as characteristic of bipolar differentiation. Beneath the blastodisc, the cytoplasm strictly joined in the yolk due to the formation of the prospective yolk syncytial layer (**Fig. 36a**).

Stage 2: two-blastomere

At 2:00, the earliest cleavage furrow divides the blastodisc into two identical blastomeres, the two-cell stage (**Fig. 36b**).

Stage 3: four-blastomere

The next cleavage furrow yields four equivalent blastomeres and is situated in a right angle to the first cleavage furrow (**Fig. 36c, 2:20**).

Stage 4: eight-blastomere

The third cleavage occurs at 2:42 and produces eight blastomeres, arranged in two parallel rows of four cells each (**Fig. 6d**).

Stage 5: early morula–16 blastomeres

The furrows of the fourth cleavage are oriented roughly horizontally to oblique (**Fig. 36e**). The germ consists of 16 blastomeres at 3:45.

Stage 6: late morula

The fifth cleavage occurs at 4:30. The following cleavage furrows developed into asynchronous producing unequal blastomeres in size and the germ comprises up to circa 32 blastomeres (**Fig. 36f**).

Stage 7: blastula

In late specimens of this stage, at 6:25, the blastodisc shows a definite pebbled emergence and its upper exterior side slightly forms a dome-shape. It is indeed distinguished from the yolk cell by a ring-like demonstrating the formation of the yolk syncytial layer (**Fig. 36g**).

Stage 8: flat blastula

The blastodisc divides into two types of cells at 7:40. The primary cell population recognized is the enveloping layer. It mostly originates from the superficial cells of the blastoderm that form an epithelial sheet with a mono-cellular layer. The deep layer of cells is located beneath the internal surface of the enveloping layer (**Fig. 36h**). Shortly after, the margin of the blastoderm expands externally and the annular groove at the blastoderm-yolk junction vanishes. This modification is the most noticeable sign indicating the start of the epiboly.

Stage 9: late blastula

At this stage the deep cells have multiplied producing a multicellular layer, observed at 8:35 to 9:00. As cleavage proceeds, cell size indeed declines while the cell number enhances leading to an advanced blastula in the limited blastodisc area. The blastoderm happens to be opaque and firm. It also shows a broad and thick belt of the external yolk syncytial layer. The animal surface of the yolk cell underlying the blastoderm is flat and most of the yolk vacuoles are located inside. Shortly after, the periphery of the blastoderm extends over the adjacent yolk margin (**Fig. 36i**).

3.3.2.4.1.2 The embryonic phase

Stage 10: epiboly, evacuation zone

Animal pole and vegetal pole are clearly separated from each other, this occurs around 16:00 h. The germ retakes on the circular shape with a lens-shaped blastoderm. The upper part is densely packed with yolk. The blastoderm covers 30 % of the yolk surface concurrently as the margin of the blastoderm is lying just beyond the lower margin of the external yolk syncytial layer. The inflated evacuation zone is covered by a single thin and clear mono-layered epidermal enveloping layer. The animal surface of the yolk sphere then turns out to be flat. The internal yolk syncytial layer gets more significant prominent beneath the extremely slim and translucent blastoderm (**Fig. 37a, 19:11**).

Stage 11: 50% epiboly

The blastoderm prolongs spreading and covering 50 % of the yolk surface. As the blastoderm extends vegetally, the external yolk syncytial nuclei moves underneath the blastoderm to distribute over the yolk mass. The margin of the blastoderm can be distinguished from the germ ring (**Fig. 37b, 25:15**).

Stage 12: embryonic shield

The margin of the blastoderm spreads over the yolk margin and a visible embryonic shield emerges as a slender bulge. This longitudinal axis of the embryonic shield will be later on recognized as the prospective embryo. The evacuation zone seems to be dislocated from the vertical axis of the yolk mass (**Fig. 37c, 27:00**).

Stage 13: early neurula

The embryonic shield happens to be elongated towards the animal pole. It is formed by a thickening of the ectodermal epithelium to form a classical neural plate. A small evacuation zone is still present, but is not seen again after this stage (**Fig. 37d, 29:15**).

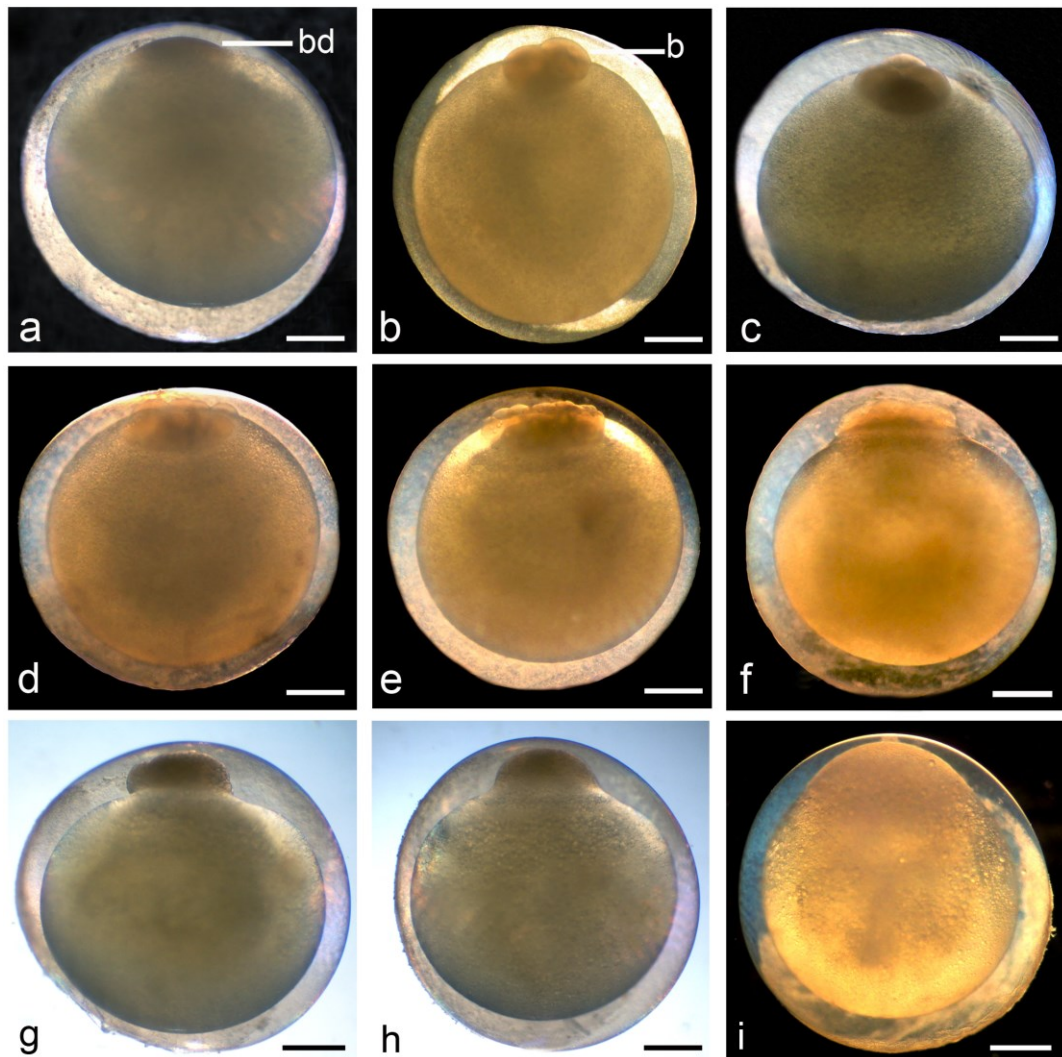


Fig. 36. Cleavage in *Notopterus notopterus* and blastulation. [a] Stage 1: one cell (zygote), 1: 10. [b] Stage 2: two cells blastomeres (b), at 2:00 [c] Stage 3: four cells, 2:20. [d] Stage 4: eight cells, 2:42. [e] Stage 5: early morula with 16 cells, 3:45. [f] Stage 6: asynchronous blastomeres up to circa 32 cells, 4:30. [g] Stage 7: blastula with compact blastodisc (bd), 6:25 [h]. Stage 8: flat blastula, note the roundish mound of the blastoderm on the top of the yolk, 7:40. [i] Stage 9: late blastula, the surface of blastoderm appears smooth but the cells are still distinct, 8:35. Scale bar = 1 mm.

Stage 14: 75% epiboly

The blastoderm has almost covered up to 75 % of the yolk surface by the end of this stage. The neural folds of the prospective head region are elevated from the epidermal yolk sac cover. A notochord can be seen at the midline of the neural plate. This stage morphologically classifies as rostral and caudal axis of the future embryo. The first three somites are also visibly recognizable from this stage onward (**Fig. 37e-f, 36:00**).

Stage 15: wedge-shaped neural plate

The blastoderm expands spreading and covering up to almost 90 % of the yolk surface, with yolk plug obviously much reduced from the previous stage. The neural plate continues to extend laterally and becomes wedge-shaped (**Fig. 37g, 39:10**).

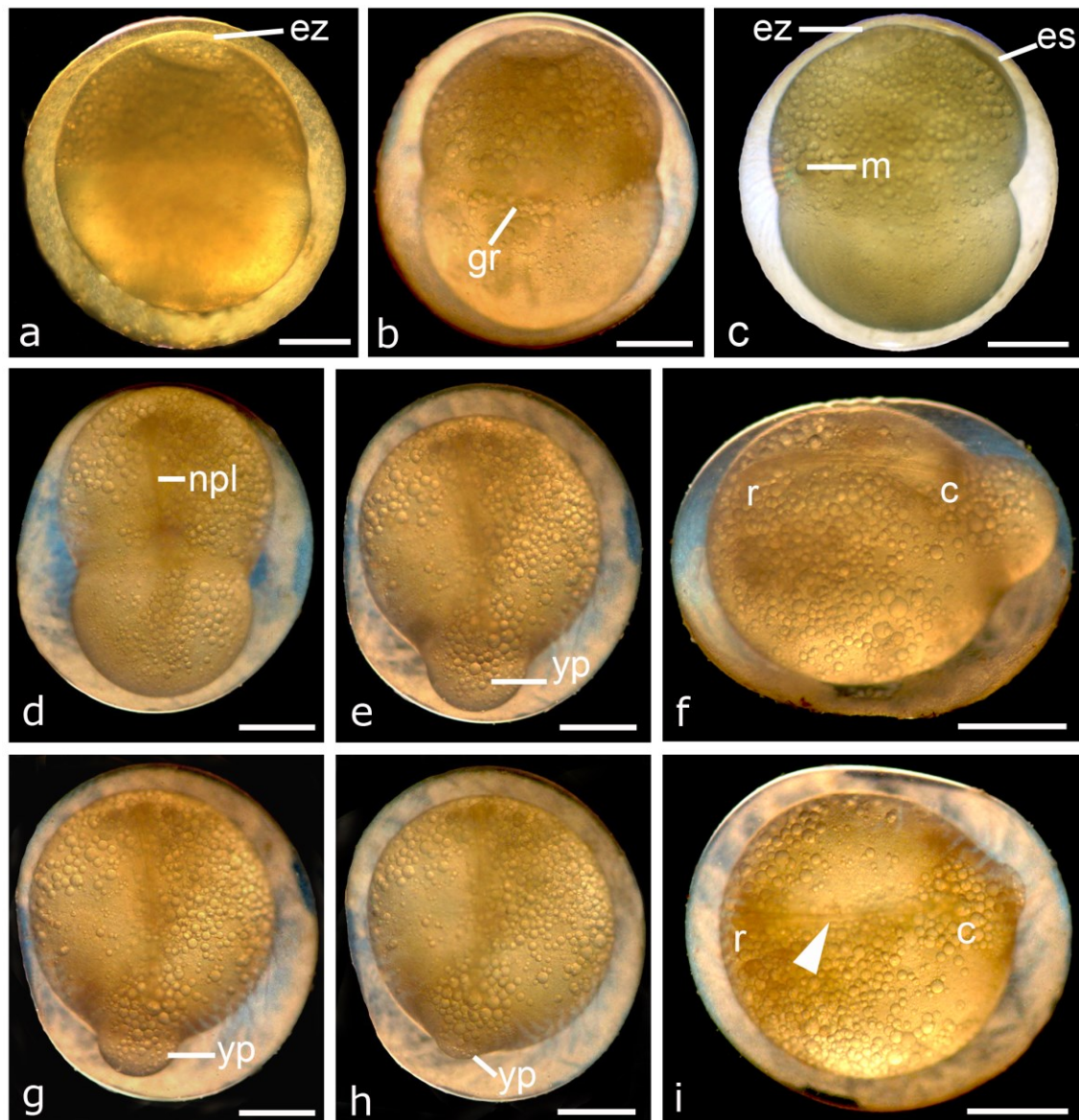


Fig. 37. Continuation of epiboly and neurulation in *Notopterus notopterus*. [a] Stage 10: evacuation zone (ez) at animal pole and the deepening of the marginal zone (arrow) between the animal pole and the vegetal pole, 19:11. [b] Stage 11: 50 % epiboly, germ ring (gr) positioned in between of animal pole and vegetal pole, 25:15. [c] Stage 12: embryonic shields on the animal pole, note the translucent evacuation zone (ez), 27:00. [d] Stage 13: onset of neurulation with vertical view, neural plate (npl), 29:15. [e] Stage 14: 75 % epiboly on vertical view, note the yolk plug is mostly constricted by the flat and short germ ring (gr), 36:00. [f] Stage 14: transversal view, note the rostral (r) and caudal (c) part can be distinguished. [g] Stage 15: 90 % epiboly; note the reduced yolk plug (yp), 39:10. [h] Stage 16: latest epiboly, note the tiny remnant of yolk plug (yp), vertical view. [i] Stage 16: transversal view showing the wedge-shaped neural plate (arrowhead), 41:35. Scale bar = 1 mm.

Stage 16: latest epiboly

The blastoderm covers almost the entire yolk surface, leaving an exposed yolk plug. The germ ring is now swollen all around. The primary yolk sac cavity extends further rostrally and caudally underneath the entire neural plate to form the segmentation cavity (Fig. 37h-i, 41:35).

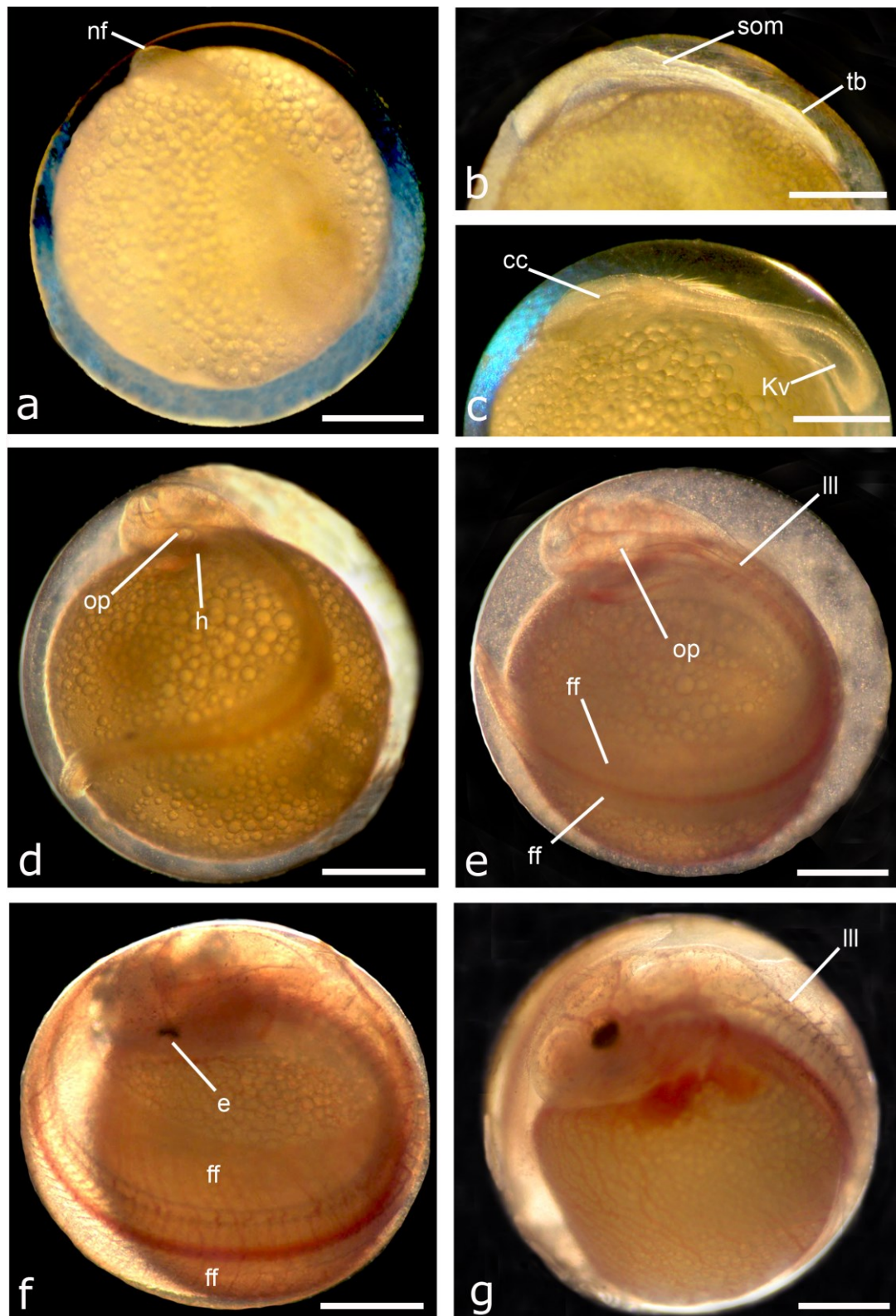


Fig. 38. Embryonic development in *Notopterus notopterus*. [a] Stage 17; spoon shaped, 48:50. Note the head cavity. [b] Stage 18: early trunk-tail bud, note the remnant of evacuation zone, 51:05. Note somites (som) and the tail bud (tb) on the posterior part. [c] Stage 19: tail-bud bent, Kupffer's vesicle (Kv) appears in the bent tail bud, 84:00. Note the corpus cerebelli (cc) around the rhombencephalon area. [d] Stage 20: otic placode (op) and heart beats (h) observed, 92:25. [e] Stage 21: fin-fold stage, 95:25. Note the lobi linea literalis (lll). [f] Stage 22: eye pigment (e), 122:05. Note the broader fin fold (ff) along the body. [g] Stage 23: "C" shaped, pre-hatching embryo, note the edge of the caudal fin, 146:50. Scale bar = 1 mm.

Stage 17: spoon-shaped

Epiboly is completed and the yolk mass is wholly wrapped with both the blastoderm and yolk syncytial layer. As neurulation proceeds, a neural groove becomes evident along the midline of the plate, with a widened, spoon-shaped depression at the anterior end. Shortly after the neural folds have approached each other along the midline, which causes the neural plate to take a dumbbell-shaped appearance. In the prospective head region, the neural folds thicken (**Fig. 38a, 48:50**).

Stage 18: early trunk-tail bud

The lateral edges of the neural plate are prominent and fold in toward the midline of the embryo, mainly in the prospective trunk-tail region. The majority of caudal portion of the neural anlage and underlying mesoderm project from the epidermal yolk sac cover form a rudimentary trunk-tail region. The embryo now has around 10–13 somites already. The earliest somatic muscular activity occurs in the posterior trunk-tail region. The observed contractions were mostly weak lateral trunk-tail flexions. A first twitch from touching the embryo was observed, which was mostly followed by a secondary reaction or several thrashes of the tail (**Fig. 38b, 51:05**).

Stage 19: tail bud-bent

This stage is also categorized by a 90° bend of the free trunk-tail. The somites and the mesoderm destined for the further differentiation of somites is fused at the midline above the spinal cord, and the mesodermal matter of the lateral plates in the head region grows dorsally. The embryo now has 19–20 somites. The observed contractions were mostly weak lateral trunk-tail flexions. Any actions from nearby will apparently provoke a particular twitch, which is then followed later on by a secondary backlash or even several thrashes of the tail. The straightening of the embryo enables then to analyse the blood circulation in more detail. The straightening of the embryo now allows following the blood circulation in more detail. The heart tube is currently visible. The caudal tail region of the embryo, in which Kupffer's vesicle is located, is separated from the yolk sac and curved downward along it (**Fig. 38c, 84:25**).

Stage 20: otic placode and heart beat

The otic placode emerges for the first time at this stage. The yolk sac is covered by a dense plexus of anastomosing subintestinal venae vitellinae. Spontaneous, vigorous, flexing movements of the body are common, which cause the embryo to rotate within the egg envelope. If illumination is changed, contraction frequency is shortly increased.

The central nervous system lays in a deep groove formed by the remarkably elevated paraxial mesoderm in the trunk-tail region and the prominent, densely packed mesoderm mass in the head region. The forming heart is seen inside the extended pericardial cavity. The blood cells were circulating through a system which included in a sequential order: a two chambered heart, a dorsal aorta from the heart to the posterior limit of the yolk, a short intestinal loop, a subintestinal-vitelline vein to the oil globule periphery, and after taking a diffuse random course across the external surface of the oil globule back to the heart. The lobi lineae lateralis becomes visible (III) (Fig. 38d, 92:25).

Stage 21: fin-fold

The first sign of the embryonic fin fold is observed here. It runs along the midline from the first somite caudally and encircles to the entire trunk-tail end. The trunk and tail region constantly perform thrashing movements. There are 28 somites visible at this stage. A dorsal aorta is discernible in the anterior body region. The entire head region has significantly enlarged and shows several new features. A lens is formed inside the eye cup, when the choroid fissure is still wide open. No pigment is discerned in the eye. The yolk sac is covered by a dense plexus of anastomosing subintestinal venae vitellinae. The membranous embryonic fin fold becomes conspicuous during the illumination (Fig. 38e, 95:25).

Stage 22: eye pigment

Diffuse pigmentation appears in the eye. A few small unbranched melanophores that contain diffuse melanine pigments are distributed all over the ectoderm of the head. The muscular contractions of the embryo have proceeded to powerful movements of the entire body by now. The vascular fin fold network extends over the entire fin fold, covering the yolk sac. Some larger venae vitellinae are established at the lateral region of the yolk mass surface. 48-52 pairs of somites could be observed (Fig. 38f, 122:05)

Stage 23: pre-hatching

The yolk cavity is enlarged beyond the entire anterior part of the embryo. The anterior part of the head is slightly undercut at its juncture with the epidermal yolk sac cover. At quiescent conditions the tip of the curved trunk-tail region reaches the vertical end of the head. This tip may touch or even pass over the head during movements. The embryos kept performing strong vigorous rotations and quivering motions. These movements are sufficient to change the orientation of the embryo inside of the egg envelope. The two columns of the presumptive corpus cerebelli fuse dorsally in the midline, forming also a transversally widened bulge behind the fissure rhombo-

mesencephalica. This bulge curves forwards, inwards, ventrally and also grows caudally, forming the primitive cerebellar cavity. The lobi lineae lateralis are very obvious and start to elevate. The embryos are in a twisted position due to their elongated body (**Fig. 38g, 146:50**).

3.3.2.4.1.3 The eleutheroembryonic phase

Stage 24: hatching

Hatching started at about 168-204 hrs after spawning and the free embryos measured about 10.5 mm. The egg envelope had lost its stability before, which could be seen by its pliable texture. The tail usually protrudes first and usually after a part of the egg envelope ruptured; the free embryos with the head part often still remain within the egg envelope for some time. The yolk sac measured around 3.8 mm in total length at this stage, situated mostly still inside the egg envelope. As the upward fin fold presents a membranous bud of the dorsal fin, the development of the dorsal fin can be followed from this stage onward (**Fig. 39a, 168:05**).

Stage 25: completed rupture

The egg envelope has completely ruptured. It took around 20 minutes until the embryo successfully detached from its egg envelope. Shortly after hatching, the embryos rest calmly and in a straight line on the ground. The eyes are completely black. The tiny, hemispherical pectoral fin projects from the dorsal epidermal yolk sac cover, and is positioned close to the heart region. The yolk sac still measures around 3 mm. There are 58–62 pairs of somites observed at this stage (**Fig. 39b, 168:25**).

Stage 26: jaw and branchial placode

The head still remains bent downwards along the yolk sphere. The first mesenchymal condensations of jaws and branchial arches can be seen between head and yolk mass. Usually the yolk mass is no longer rounded, but has become egg shaped with a narrow posterior end. The yolk sac measures around 2 mm and the free embryos usually measure 11 mm at this stage. When the freshly hatched embryo is stimulated, it makes rapid, striking movements, especially with its trunk and tail, through which it whirls around in circles. The early free embryos are still too heavy for directed movements (**Fig. 39c, day 8**).

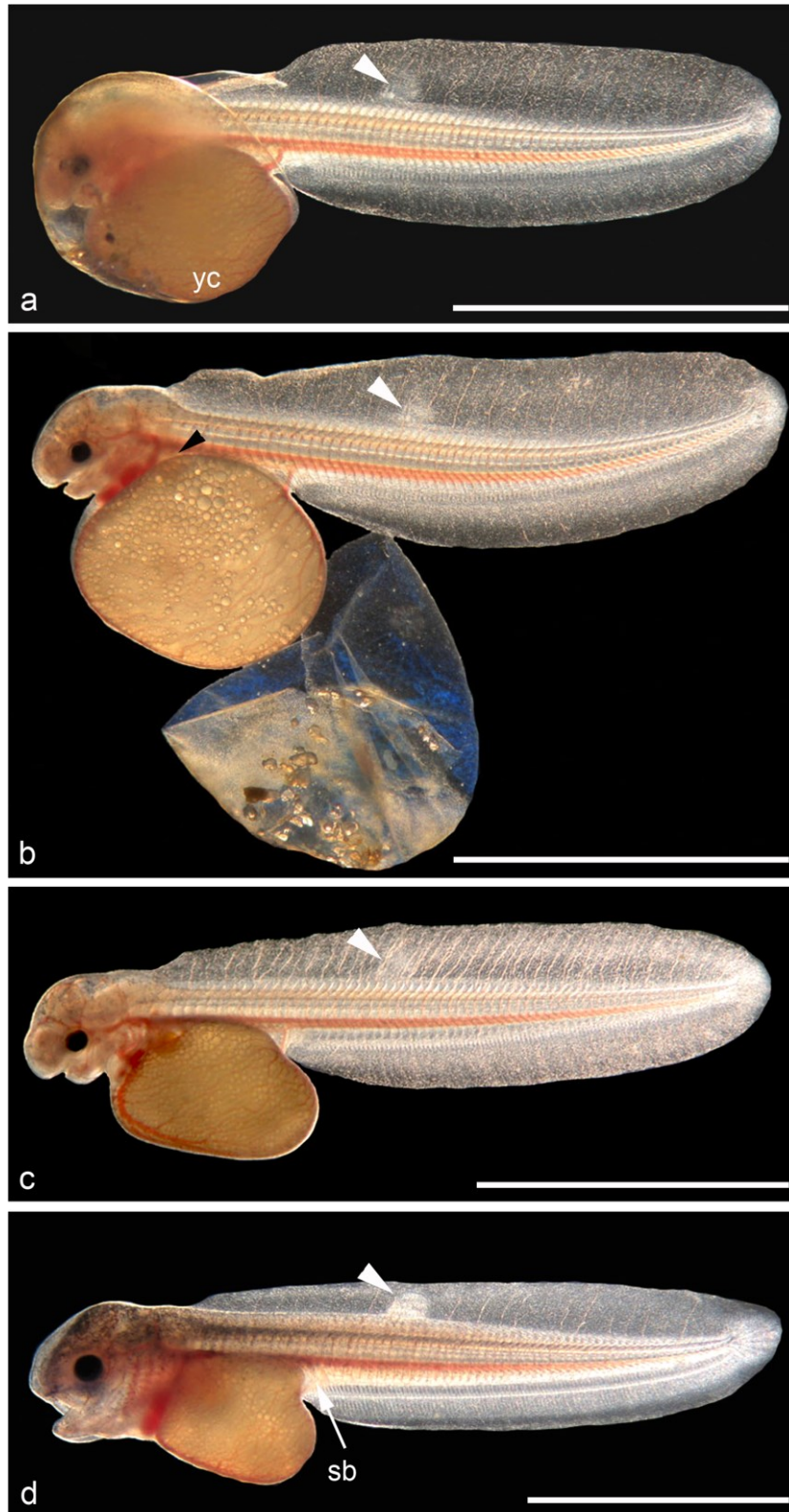


Fig. 39. Hatching process and free embryonic in *Notopterus notopterus*. [**a**] Stage 24: just hatched-after breaking the weak egg envelope, caudal part has relieved, anterior part still attached to the remnant of the egg-envelope, note the emergence of dorsal and anal fins. Yolk sac (yc) still covered with egg envelope, 168:05. [**b**] Stage 25: rupture the complete egg-envelope and free anterior part of the embryo, 168:25. Note the tiny pectoral fin bud, black arrowhead. [**c**] Stage 26: jaw and branchial placode, day 8. [**d**] Stage 27: mouth opening, day 10. Note the emergence of swim bladder (sb), arrow. White arrowheads depict gradual development of dorsal fin. Scale bar = 5 mm

Stage 27: mouth opening

Some evident changes in the outer appearance and in the structure of the head can be easily seen. The head process has already undergone a significant lift. Several structures are clearer defined. In particular, the heart is beginning to descend into the pericardial cavity. The anlagen of the dorsal and anal fins appear simultaneously as denser concentrations of mesenchyme in the fin fold. The formation of the first rays in the caudal fin is evident from this stage onward. The future swim bladder can be pointed out. Melanophores spread out significantly not only on the head part but also appear for the first time on the trunk and tail part. The head lift and further growth and straightening of the head exposes the underside of the head.

The lower jaw has straightened forward. Mouth opening shows a “>” shaped with clear separation between upper and lower jaws. The central lepidotrichial rays began to segment. The yolk sac measures around 1.4 mm and the embryos measure 12 mm (**Fig. 39d, day 10**). The tiny pectoral fin bud becomes ellipsoid in shape due to its dorsocaudal prolongation, as seen detail in **Figure 40**. The pectoral fin is clearly larger than the eye now.

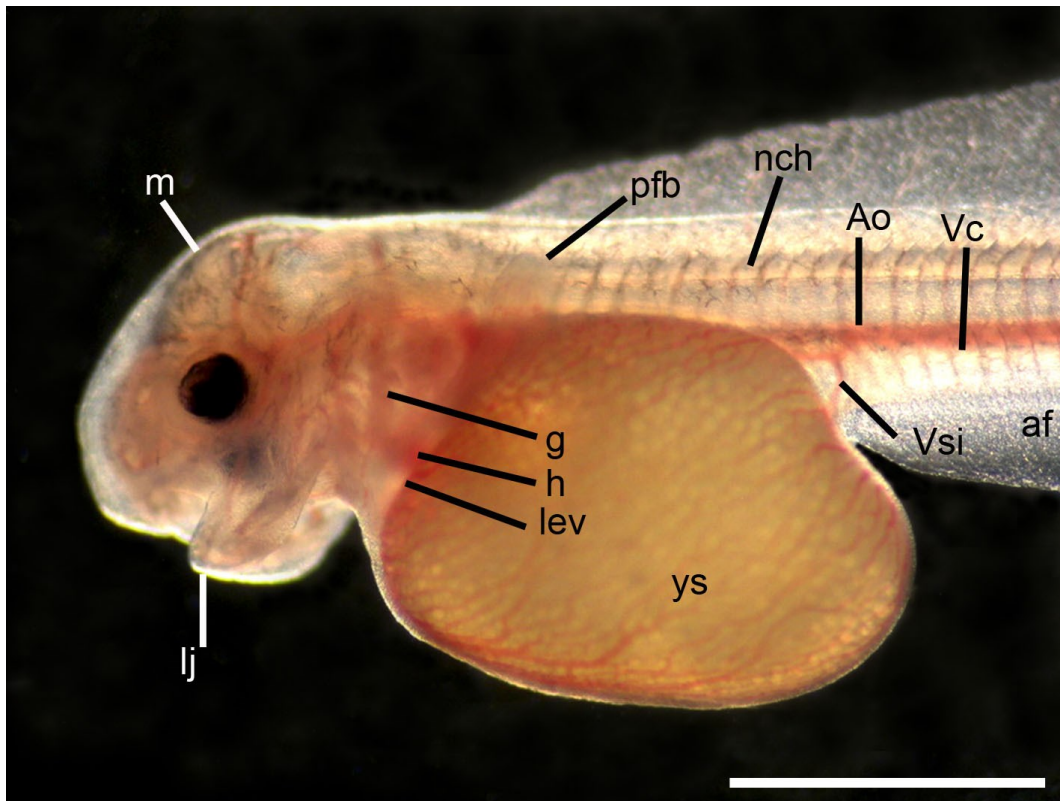


Fig. 40. Pectoral fin buds (pfb) in *Notopterus notopterus*, larger magnification - day 9. m- mesencephalon; lj- lower jaw; nch – notochord; Ao – aorta dorsalis; Vsi – vena subintestinalis; Vc – vena caudalis; af – anal fin; g- gills; h – heart; lev – left efferent vena vitellina; ys – yolk sac. Scale bar = 2 mm.

Stage 28: commence on median fin-fold regression

The dorsal and anal fins lengthen over the embryonic fin fold. The continued regression of the median fin fold allows determining the contour of the dorsal, anal, and caudal fins. The pectoral fin anlage now is circular with an enlarged and indented rim. The haemal and hypural processes are covered by a few muscles. From this stage onward, the embryos become increasingly more mobile. The embryo is now capable of completely lifting its head and actively closing or opening its mouth. The upwards flexion of the end of the notochord has reached its final shape, perpendicular to the longitudinal axis of the embryo. The embryos measured 13.6 mm and the capacity of the yolk-sac had decreased significantly (**Fig. 41a, day 12**).

Stage 29: late embryonic

The embryonic fin fold has significantly shrunk in height, especially in the dorsal part. The embryos measured 14.9 mm. The yolk sac remains visible. The pectoral fins are now fully formed with 6 segmented lepidotrichial rays each. They are functional and used as in other teleostean larvae for propulsion. In this stage, first movements of active breathing of the gill cover were observed (**Fig. 41b, day 14**).

3.3.2.4.2 The larval period

Stage 30: exogenous feeding

The first stage of the larval period is characterized by exogenous feeding concurrent with endogenous nutrient-utilization. The yolk sac remnant is still present and almost absorbed. The swim bladder appears tube-shaped and continues to develop from this stage onward. The dorsal fin has evolved showing newly developed fin-rays. The final shape of the caudal fin is reached by the conspicuous constriction and complete regression of the remaining fin fold at the caudal end of the peduncle. Melanophores spread over the exposed integument. Pectoral fins are well developed and already mobile. The depicted individual larva at this stage measures 16.2 mm (**Fig. 41c, day 17**).

Stage 31: developed-eye

The swim bladder starts to elongate dorso-rostrally in order to connect with the otic vesicle. The yolk sac remain now completely absorbed. From this stage onward, the larvae are very mobile and actively hunting for higher food densities. The ring of the eye has developed well and the larvae start to swim, although only on the bottom. The swim bladder has elongated a bit more and appears thinner. An intensely yellow mass

filled the gut. The total length of the depicted individual larva is 21 mm (**Fig. 41d, day 24**).

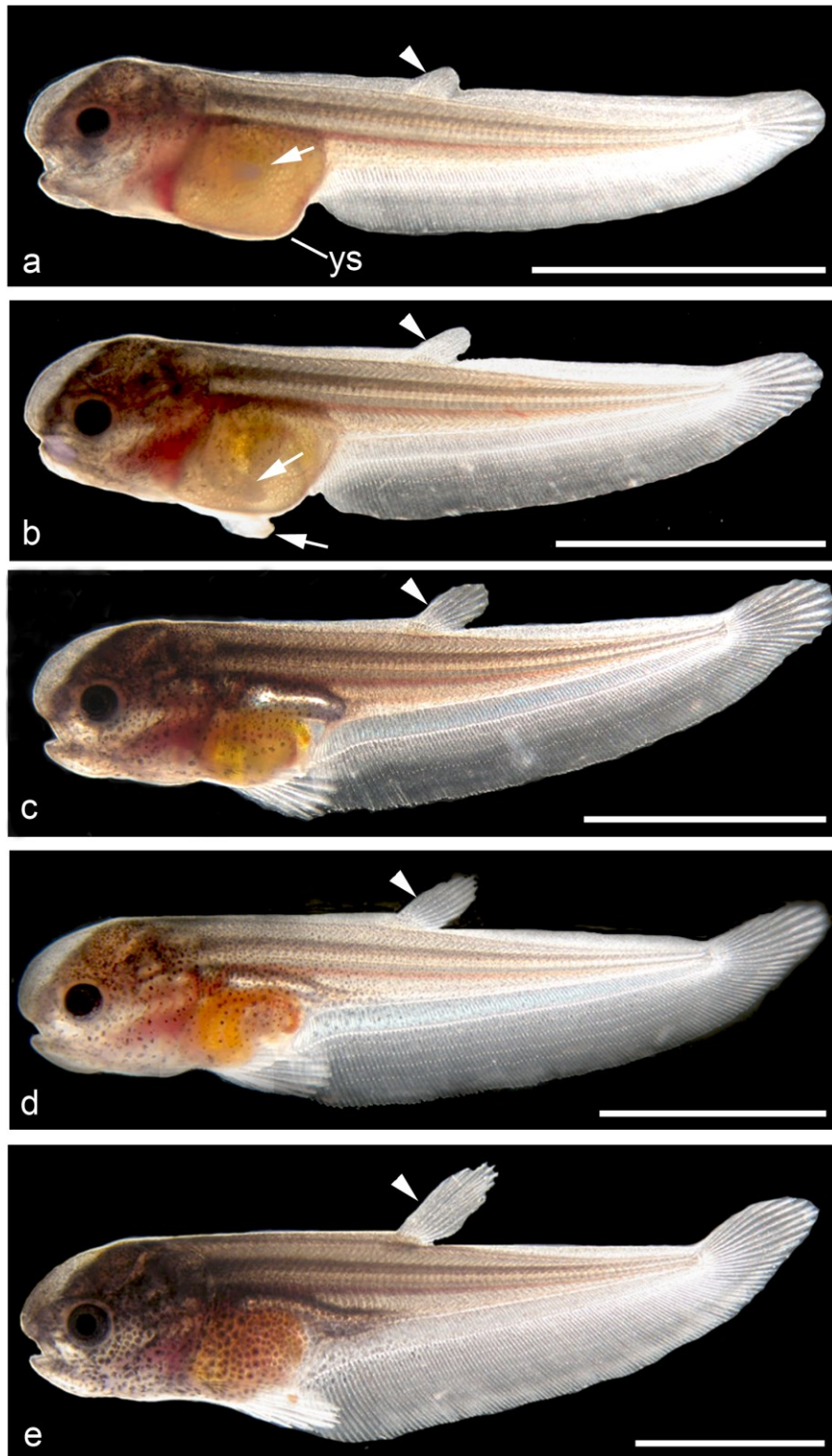


Fig. 41. Late embryonic and larval development in *Notopterus notopterus*. [**a**] Stage 28: pronounced regression of median fin fold, day 12. Note almost complete reduced yolk sac (ys). Note the pectoral fin, arrow. [**b**] Stage 29: late embryonic phase, day 14. [**c**] Stage 30: developed-swim bladder, day 17. [**d**] Stage 31: pigmented iris is very distinct, day 24. [**e**] Stage 32: caudal lobe separation, day 36. Arrowheads depict gradual development of dorsal fin. Scale bar = 5 mm.

Stage 32: caudal lobe separation

The dorsal and anal fins separate from the peduncular fin fold remain and the asymmetry of the caudal fin has become more conspicuous. The larvae usually measure 24 mm or more at this stage (**Fig. 41e**, day 36). The density of melanin has been multiplied on the entire body, especially underneath the pectoral fins and the area above the intestine. The development of the dorsal and caudal fin is presented largely magnified and in detail in **Figure 42** and **43** below.

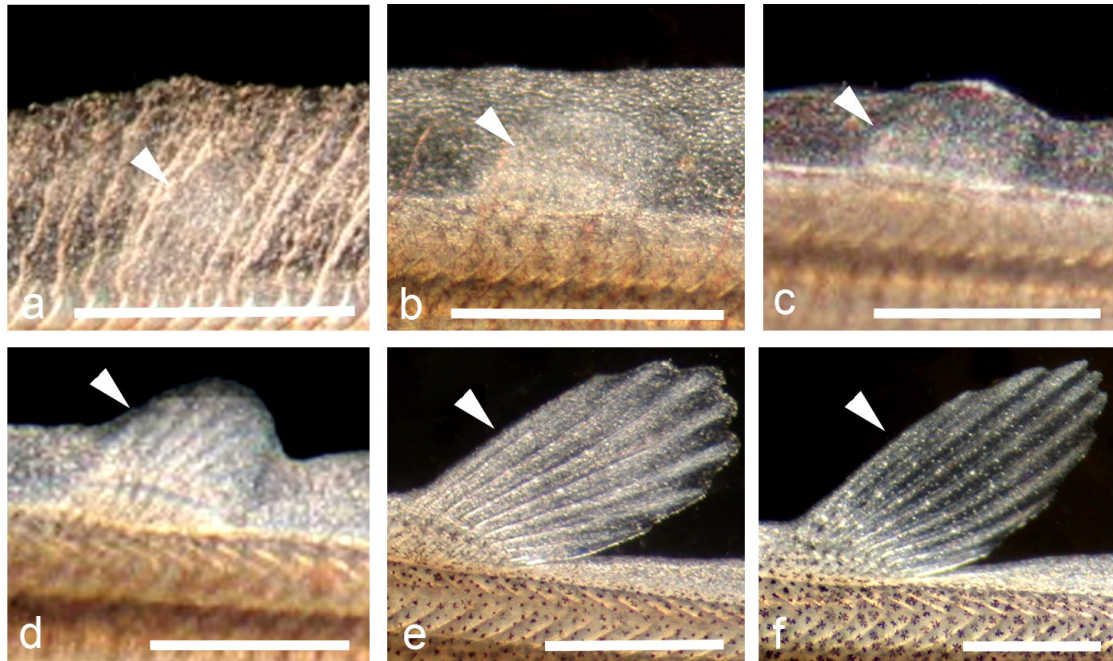


Fig. 42. Sequence of the development of the dorsal fin starting from hatching until the end of larval period in *Notopterus notopterus*. [a] shortly after hatching, day 7. [b] Dorsal fin bud slightly appeared, day 8. [c] Distinct dorsal fin and regressing of fin fold, day 11. [d] Fin rays on dorsal fin can be seen, day 12. [e] Well-developed dorsal fin with 9 fin-rays, 17 days. [f] Dorsal fin in a juvenile, 36 days. White arrowhead depicts the gradual development of dorsal fin. Scale bar = 2 mm.

3.3.2.4.3 The juvenile period

The juvenile period of *Notopterus notopterus* comprises five stages as follows:

Stage 33: juvenile – 1

This stage can be clearly characterized as a transition mark from the larval period to the juvenile period by its profound change in the pigmentation pattern. The embryonic fin folds are no longer visible. The swim bladder elongates further and is densely covered with melanophores. The lateral line of the body becomes visible, starting right behind the head region at this stage. The formation of tube-like anterior nostrils is also starting from this stage onward. The body grows significantly in height and melanophores become denser also in the caudal part. Silver-coloured scales appeared on the midline of

the anterior part of the trunk and at the base of the pectoral fin and caudal fin. The expansion of the stomach and the intestine can be clearly seen. The typical early juvenile measures now 29 mm (**Fig. 46a, day 52**).

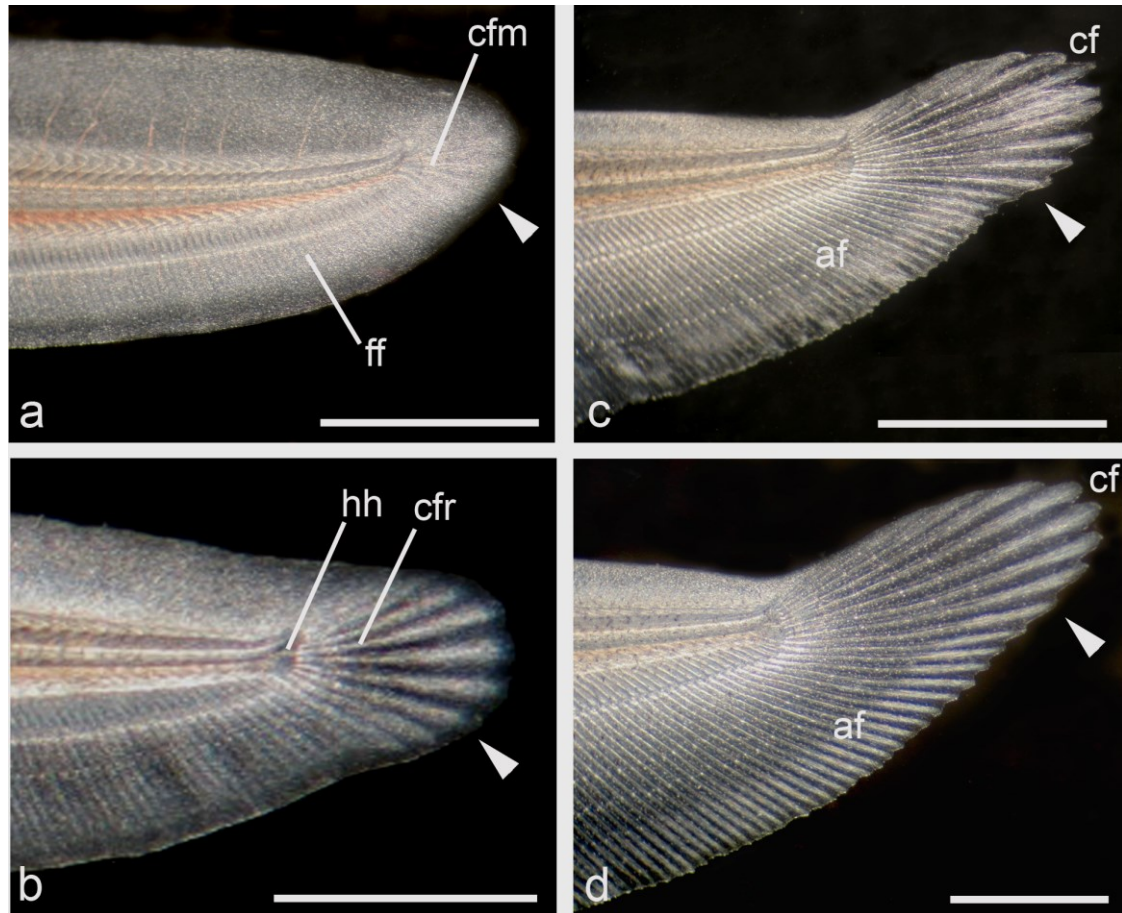


Fig. 43. Sequence of caudal fin development starting from hatching until larval period in *Notopterus notopterus*. [a] shortly after hatching, day 7. [b] Haemal and hypural plate (hh) emerged. Note the slight regression of fin fold, day 10. [c] Completely disappeared fin fold with 10 caudal fin rays, day 20. [d] Well-developed caudal fin in a juvenile, day 36. cfm- caudal fin mesenchyme; ff- fin folds; cfr – caudal fin rays; cf – caudal fin; af – anal fin. Scale bar = 2 mm.

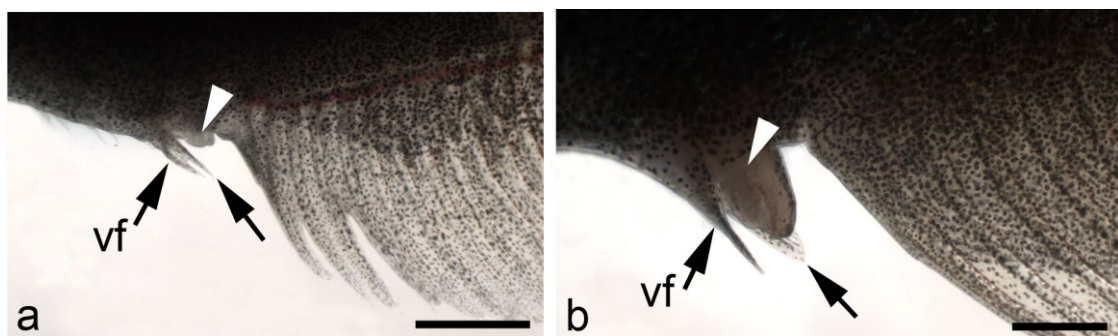


Fig. 44. Two sequences of female's genital papilla in *Notopterus notopterus* in comparable magnification. [a] The emergence of genital papilla in female with total length 52 mm. [b] Genital papilla of female with total length of 145 mm. Note the significant growth of genital papilla (white arrowhead) in between ventral fins (arrow) and anal fin. Scale bar = 2 mm.

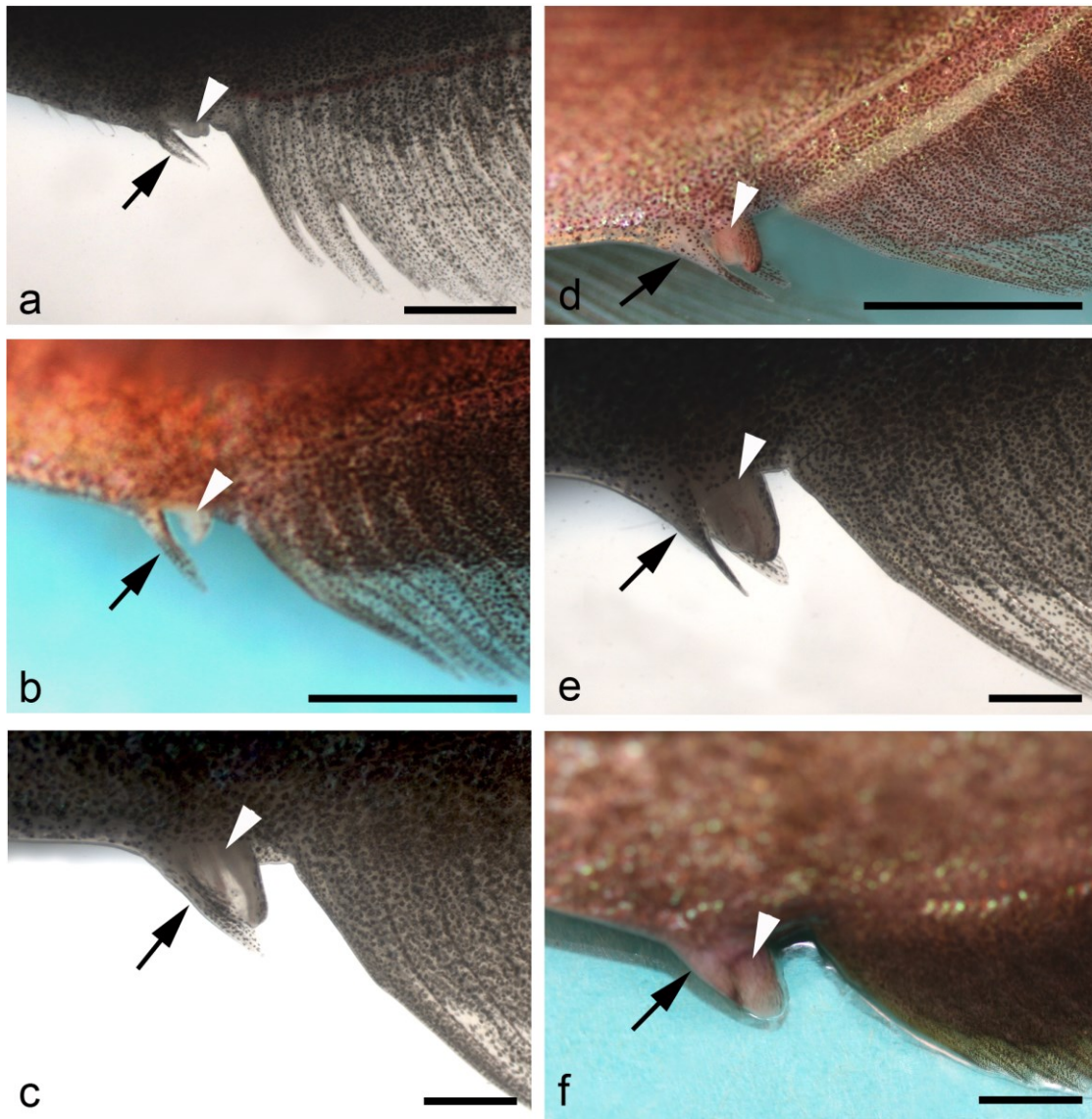


Fig. 45. Complete development of female's genital papilla in *Notopterus notopterus*, seen in various magnified photographs. [a] The emergence of genital papilla, total length (TL) 52 mm and total weight (TW) 1.77 g, day 83. Note the developed pair of ventral fins. [b] Female's genital papilla with TL 100 mm, 5 months. [c] TL=124 mm, 8 months. [d] TL= 136 mm, 12 months. [e] TL=145 mm and TW= 28.5 g, in 18 months old. [f] Mature genital papilla obviously longer than the pelvic fins, TL= 230 mm, captured at 24 months old. Note the significant growth of genital papilla (white arrowhead) in between of ventral fins (arrow) and anal fin. Scale bar= 2 mm (a, c, e); 5 mm (b, d, f).

Stage 34: juvenile - 2

At this stage the body has fully changed its colour into vertically arranged dark brown stripes. There is a slight black colour at the outer part of the eye. This typical juvenile-2 measures 34 mm (**Fig. 46b, day 70**)

Stage 35: juvenile - 3

The body shows much stronger dark brownish stripes and commencing elongation of tubular nostrils is apparent at the snout. The genital papilla emerges for the first time. The depicted individual measures 46 mm TL (**Fig. 46c, day 80**).

Stage 36: juvenile - 4

There is once again an obvious change in the pigmentation pattern from the previous stage. The striped, dark-brown colouring has disappeared. The whole body turns into brownish-gold and scales are externally visible from this stage onward. The pale remains of dark brown stripes can still be recognized. The pelvic fins start to emerge at this stage and the depicted individual measures 76 mm TL (**Fig. 47a, day 92**). Series development of genital papilla in a female is shown in **Figure 44** and **45**.

Stage 37: juvenile - 5

The dorsal fin loses its trapezoidal shape and forms into the triangular shape of the adult. The specimens have attained the general body profile of the immature adult now. The genital papilla is clearly recognisable behind the elongated pelvic fins. The depicted late juvenile individual measures 145 mm TL (**Fig. 47b, 18 months**).

3.3.2.4.4 The adult period

Stage 38: adult

The head shape and body pigmentation are different from the late juvenile. From this stage onward, the genital papilla of the mature can be easily differentiated between male and female by its form. The female's genital papilla is whitish, broader and shorter, whereas the male's is yellowish, thinner and longer. Gonad maturation can be easily recognized by the swollen abdomen and increase of appetite starting at the age of 24 months. The illustrated individual adult measures 275 mm TL (**Fig. 47c, 30 months**). A glance of all development stages in *Notopterus notopterus* is presented in **Table 6**.

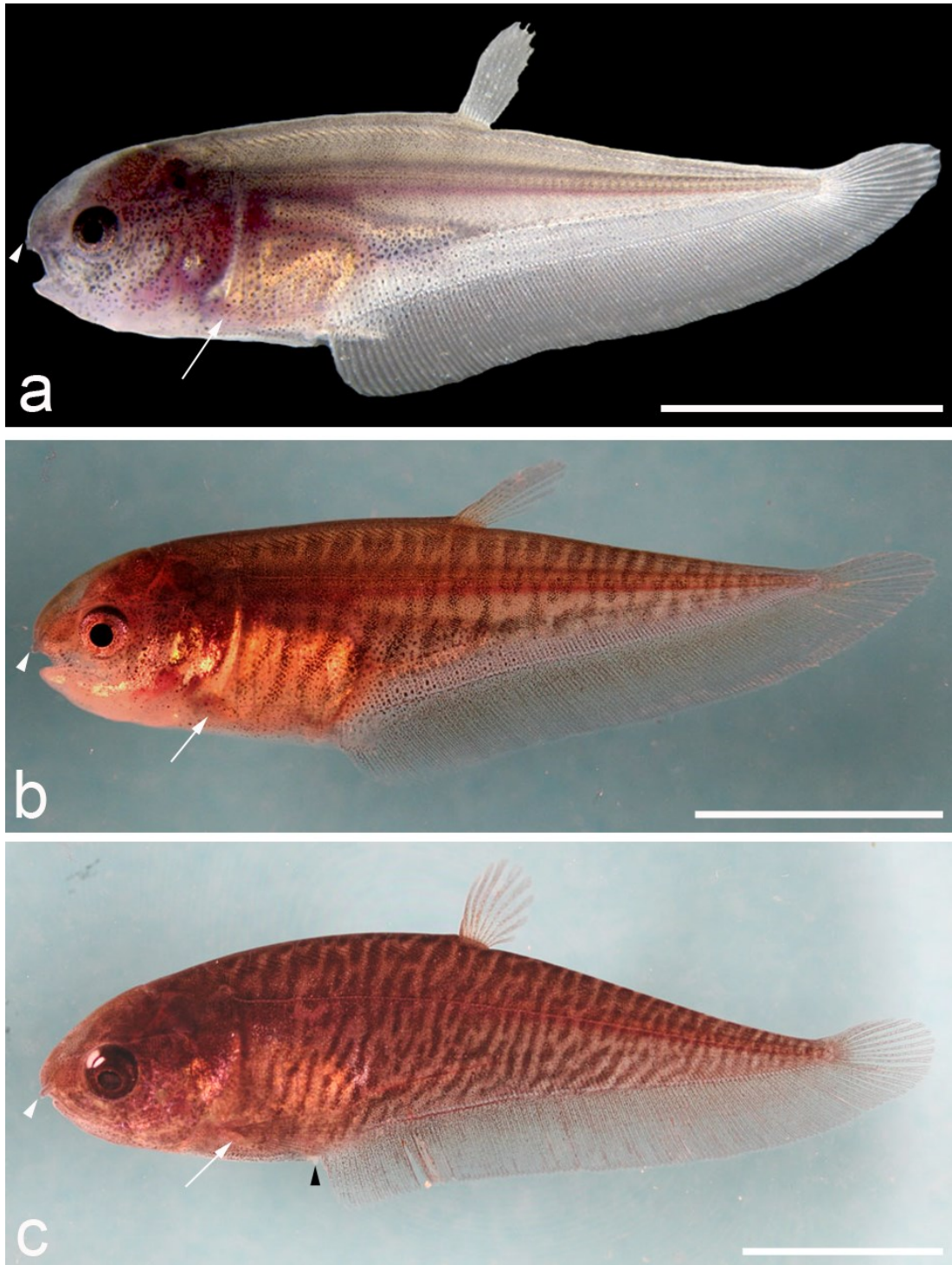


Fig. 46. Juvenile transformation in *Notopterus notopterus*. [**a**] Stage 33: early juvenile in larger magnification, note the appearance of lateral line and short tubular nostrils, day 52 [**b**] Stage 34: stripe colored body of juvenile, day 70. [**c**] Stage 35: strong stripe coloured alongside the body, note developed tentacles of the anterior nostrils, day 80. Arrow points the base of well developed pectoral fin. White arrowheads show the anterior nostrils. Black arrowhead points the genital papilla. Scale bar = 10 mm.

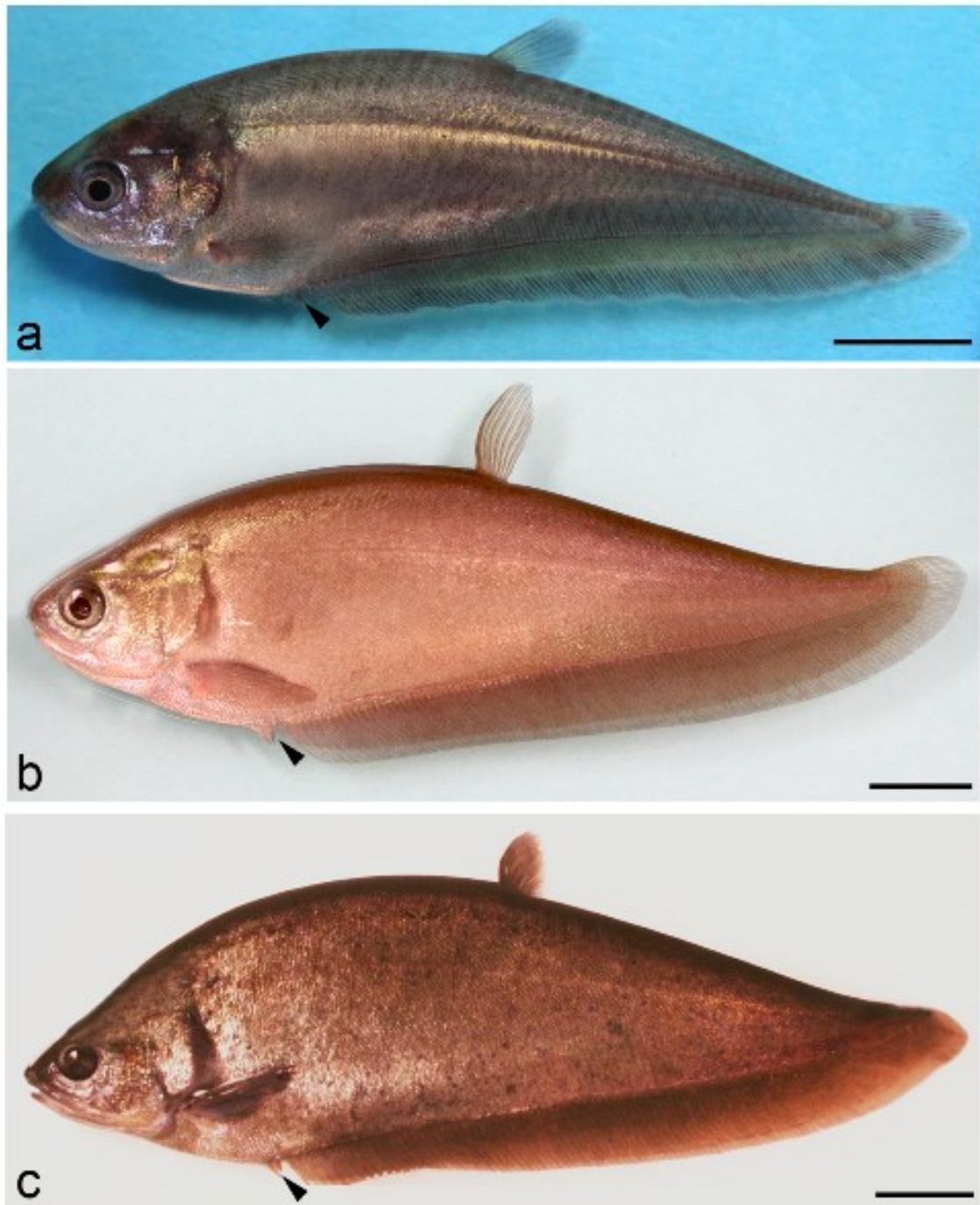


Fig. 47. Late juvenile to the maturation stage in *Notopterus notopterus*. [**a**] Stage 36: appearance of ventral fin, black arrowhead, day 92. [**b**] Stage 37: late stage of juvenile with total length of 145 mm, note the change in colour and the emergence of scales, 18 months. [**c**] Stage 38: adult male with total length 275 mm, captured at age of 30 months. Note the ventral fin (arrowhead) longer than the genital papilla. Scale bar = 10 mm (a); 20 mm (b); 30 mm (c).

Table 6. Overview of developmental stages of *Notopterus notopterus* (27 °C). Determination of periods after Balon (1975)

Period:	Phase	Stage	Age (hrs:min)	Characteristics	Figure
Embryonic	Cleavage	1	1:10	Zygote-1 cell	36a
		2	2:00	2 Blastomeres	36b
		3	2:20	4 Blastomeres	36c
		4	2:42	8 Blastomeres	36d
		5	3:45	Early morula	36e
		6	4:30	Late morula	36f
		7	6:25	Blastula	36g
		8	7:40	Flat blastula	36h
		9	8:35	Late blastula	36i
	Embryonic	10	19:11	Evacuation zone	37a
		11	25:15	Epiboly 50%	37b
		12	27:00	Embryonic shields	37c
		13	29:15	Early neurula	37d
		14	36:00	Epiboly 75%	37e-f
		15	39:10	Wedge-shaped neural plate	37g
		16	41:35	Latest epiboly	37h-i
		17	48:50	Spoon-shaped	38a
		18	51:05	Early trunk-tail bud	38b
		19	84:00	Tail bud bent	38c
		20	92:25	Otic placode and heart beat	38d
		21	95:25	Fin fold	38e
Larval	Eleutheroembryonic	22	122:05	Eye pigment	38f
		23	146:50	Pre-hatching stage	38g
		24	168:05	Hatching stage	39a
	Protopterygiolarval	25	168:25	Completed rupture	39b
		26	day 8	Jaw and branchial placode	39c
		27	day 10	Mouth opening	39d
		28	day 12	Emergence of finfold regression	41a
	Pterygiolarval	29	day 14	Late embryonic	41b
		30	day 17	Swim bladder	41c
		31	day 24	Developed eye	41d
Juvenile		32	day 36	Caudal lobe separation	41e
		33	day 52	Juvenile 1	46a
		34	day 70	Juvenile 2	46b
		35	day 80	Juvenile 3	46c
		36	day 92	Juvenile 4	47a
Adult		37	18 months	Juvenile 5	47b
		38	30 months	Adult	47c

3.4 Osteoglossidae

3.4.1 *Osteoglossum bicirrhosum*

3.4.1.1 External morphology and *in situ* condition of dissected gonads

Figure 48 and **49** illustrate the Silver Arowana (*Osteoglossum bicirrhosum*) and Blue Arowana *Osteoglossum ferreirai*, respectively. Male and female of both species do not show a clearly visible sexual dimorphism. The female can only be recognized by its swollen belly during the maturation phase. *Osteoglossum* ssp. has a pair of barbels located on the lower jaw. In both species strong and hard cycloid scales cover the entire body.



Fig. 48. A female of *Osteoglossum bicirrhosum* [a] of 63 cm total length and 59 cm standard length. [b] In the head the diameter of the eye is indicated as 18 mm, the characteristic barbels, and a large scale taken out from the dorsal part of the body. [c] The posterior part with caudal, anal and dorsal fins in gold or brown. [d] *In situ* condition of dissected abdomen. [e] Single ovary on the left side of the body cavity is densely covered by fat, situated on the right corner of the body cavity, arrow. Scale bar = 10 cm.

The silvery-brown *Osteoglossum bicirrhosum* (Fig. 48a) shows an elongate body covered with large scales (Fig. 48b) as well as the long anal fin together with the caudal fin and dorsal fin (Fig. 48c). The barbels comprise 20 mm in length in the shown adult specimen (Fig. 48d). *Osteoglossum ferreirai* (Fig. 49a) is similar in external morphology to *O. bicirrhosum*, except for the anal fin, caudal and dorsal fins, which are blue (Fig. 49b, c). The barbels of the Blue Arowana, presented in Fig. 49d, are 18 mm in length. *In situ* conditions (Fig. 48e, 49e) of female specimens of both species reveal one single ovary, which is located in the left hind corner of the body cavity. Among 16 specimens (11♀, 5♂), total gonad weight (TW) of the females ranged from 1.7 to 48.1 g, with total length (TL) varying from 51.5 to 67 cm, whereas total weight of the males testes varied from 0.3 to 0.7 g, with TL ranging from 40.2 to 60 cm.



Fig. 49. A female of *Osteoglossum ferreirai* (Blue Arowana). [a] 60 cm total length and 56.5 cm standard length. [b] Blue colour of anal, [c] caudal and dorsal fin can be clearly seen. [d] *In situ* condition of opened abdomen. [e] Ovary is filled mostly with stadium IV oocytes. Scale bar = 10 cm.

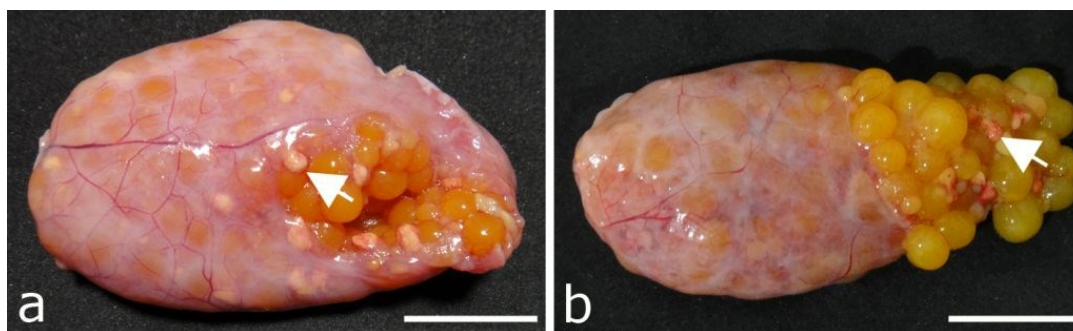


Fig. 50. Two ovaries of *Osteoglossum bicirrhosum*. [a] with total gonad weight (TW) 31.5 g and [b] *Osteoglossum ferreirai* with total gonad weight 39.8 g. Both ovaries clearly contain large numbers of oocytes in different stages of development accompanied by some fat, arrow. Photos were taken shortly after dissection and measurement of gonad weight. Scale bar = 2 cm.

3.4.1.2 Gonad of Silver Arowana and Blue Arowana

The gonads of dissected *O. bicirrhosum* (Silver Arowana) and *O. ferreirai* (Blue Arowana) are illustrated in **Figure 50**. The ovaries were filled with oocytes in different stages (stadium I to stadium IV). Maturity coefficients (MC) of these two ovaries are 2.75 % (*O. bicirrhosum*, TL = 63 cm) and 3.4 % (*O. ferreirai*, TL= 60 cm), respectively.

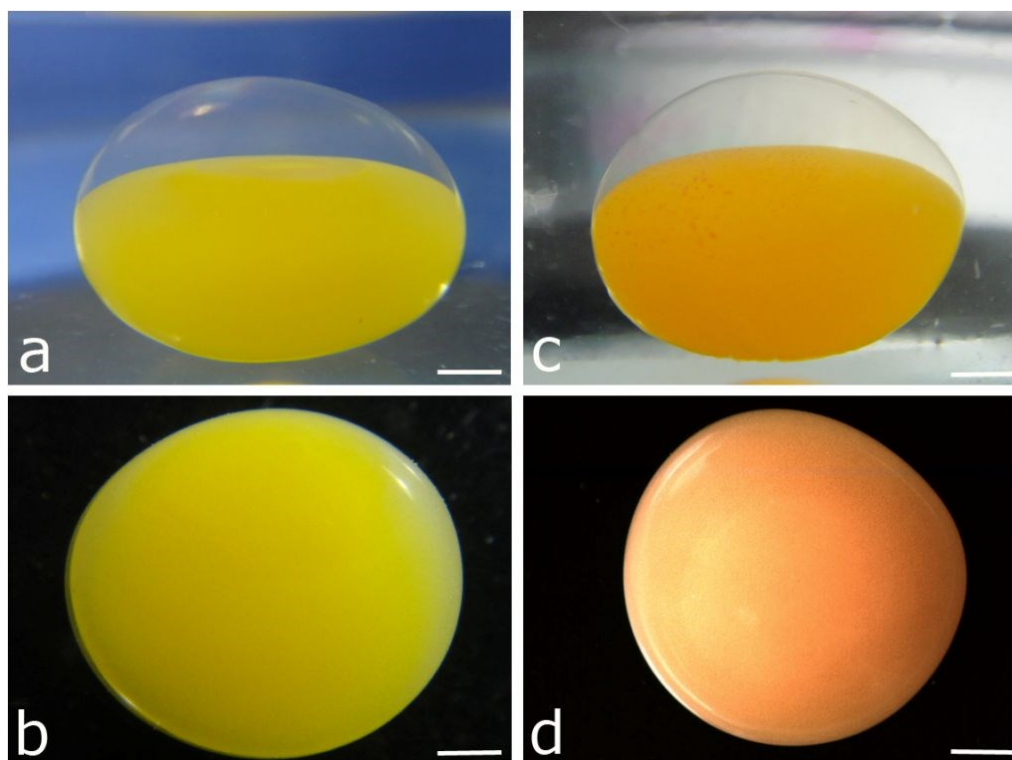


Fig. 51. [a-d] Freshly collected non-adhesive eggs of *Osteoglossum bicirrhosum*. Two different colours of eggs, [a, b] yellow and [c, d] orange, were found during this study of eggs with the same diameter of 12 mm. The eggs with transparent chorion, [a, c] viewed from the side of a Petri dish and [b, d] others viewed from the top. Scale bar = 2 mm.

3.4.1.3 Egg of Silver Arowana

Table 7 presents the list of visited farms of Silver Arowana within five months including the numbers of collected, fresh eggs, numbers of collected eggs with embryo and numbers of collected juveniles with the yolk sac, taken directly from the male's mouth. Egg numbers per collecting varied from 23–220. The eggs of *O. bicirrhosum* ranged from 11 to 12 mm in diameter with a total weight of 0.9–1.08 g. They are roundish, non-adhesive and easily sink to the bottom (**Fig. 51**). The eggs are covered with a thin, transparent and fairly robust egg envelope, which encloses a narrow perivitelline space. As **figure 51a** and **51c** illustrate, there were two different colours of eggs found: orange and yellow. The phenomenon of yellow coloured eggs was mostly found in female specimens younger than 28 months, whereas in specimens older than 28 months the gonads contained mostly orange coloured eggs.

Table 7. All visited farms of *Osteoglossum bicirrhosum* in Florencia and other cities nearby together with the numbers of collected fresh eggs (♂1), eggs containing embryo (♂2) and juveniles with yolk sac (♂3 and ♂4) taken directly from male's mouth

No	Name of Farm	Date	♂1	♂2	♂3	♂4
1	Hermes	14.01.2011	0	0	0	0
2	Navaja	16.01.2011	0	0	0	0
3	Diego Palace	19.01.2011	0	0	0	0
4	Luis Helga	21.01.2011	45	10	0	0
5	Christian	27.01.2011	0	0	0	0
6	Ricarvte Silva	30.01.2011	0	0	0	0
7	David Garcia	01.02.2011	0	0	0	0
8	Navaja	02.02.2011	220	72	0	0
9	Navaja	09.02.2011	0	0	0	0
10	Navaja (09:30 am)	19.02.2011	27	103	80	0
	Navaja (17:25 pm)	19.02.2011	23	21	0	0
11	Navaja	22.02.2011	0	0	0	0
12	Luis Helga	28.02.2011	0	0	0	0
13	Diego Palace	12.03.2011	0	0	0	0
14	Alex	13.03.2011	40	6	0	0
15	Navaha	22.03.2011	145	0	0	0
16	Viktor	28.03.2011	96	0	0	0
17	Doncello-Acuica	17.04.2011	0	31	35	33
18	Hermes	24.04.2011	112	0	0	0
	Hermes	24.04.2011	106	0	0	0
19	Navaja	01.05.2011	0	0	0	0
20	Diego Palace	14.05.2011	0	0	0	0
21	Victor	16.05.2011	25	0	0	0

3.4.1.4 Development

The exact time of fertilization is unknown since the eggs were collected directly from the male's mouth. Therefore the age given in this study was calculated from the time, when the eggs had been collected in the farm. Eggs and embryos were incubated and raised at 28 °C. The terminology of the development of *Osteoglossum bicirrhosum* follows the classifications of Balon (1975). There are five periods: the embryonic period (I), the larval period (II), the juvenile period (III), the adult period (IV) and the senescent period (V). The larval period will not be presented, since most likely this species does not undergo this particular period. Similar to *Notopterus notopterus* the adult period is also excluded in this work. Time of egg collection is defined as time zero in age determination; it is presented as hrs:min or days.

3.4.1.4.1 The embryonic period

3.4.1.4.1.1 The embryonic phase

Stage 1: freshly captured eggs

Newly spawned eggs were observed circa 3:00 after collecting the eggs from the farm. The eggs are non-sticky, translucent, and roundish in shape and possess a large yolk-sac. Due to its large yolk it is really difficult to position the egg properly (**Fig. 52a**).

Stage 2: neural plate

Much later cleavage and gastrulation is followed by neural plate formation at 8:00, which extends laterally and becomes (**Fig. 52b**) a distinct neural plate observed as seen in **Figure 52c**. A trunk tail mound was evident almost at the same age. There was an aggregation of cells in the head area.

Stage 3: onset somatogenesis

As neurulation continues, a neural groove becomes obvious and lengthens along the midline of the plate, with a widened, spoon-shaped head formation at the anterior end. The location of the future eye can be seen. The head starts to develop at this stage. Brain regionalization, a lateral symmetrical thickening of the neural folds, appears in the head region. The head area was swollen, showing first indications for developing brain parts. It filled the perivitelline space and touched the membrane next to the micropyle. The presumptive heart tube is already positioned on the right base of the anterior of the body. No blood elements were visible. The neural plate is longer and broader than in previous stage (**Fig. 53a-c, 21h**).

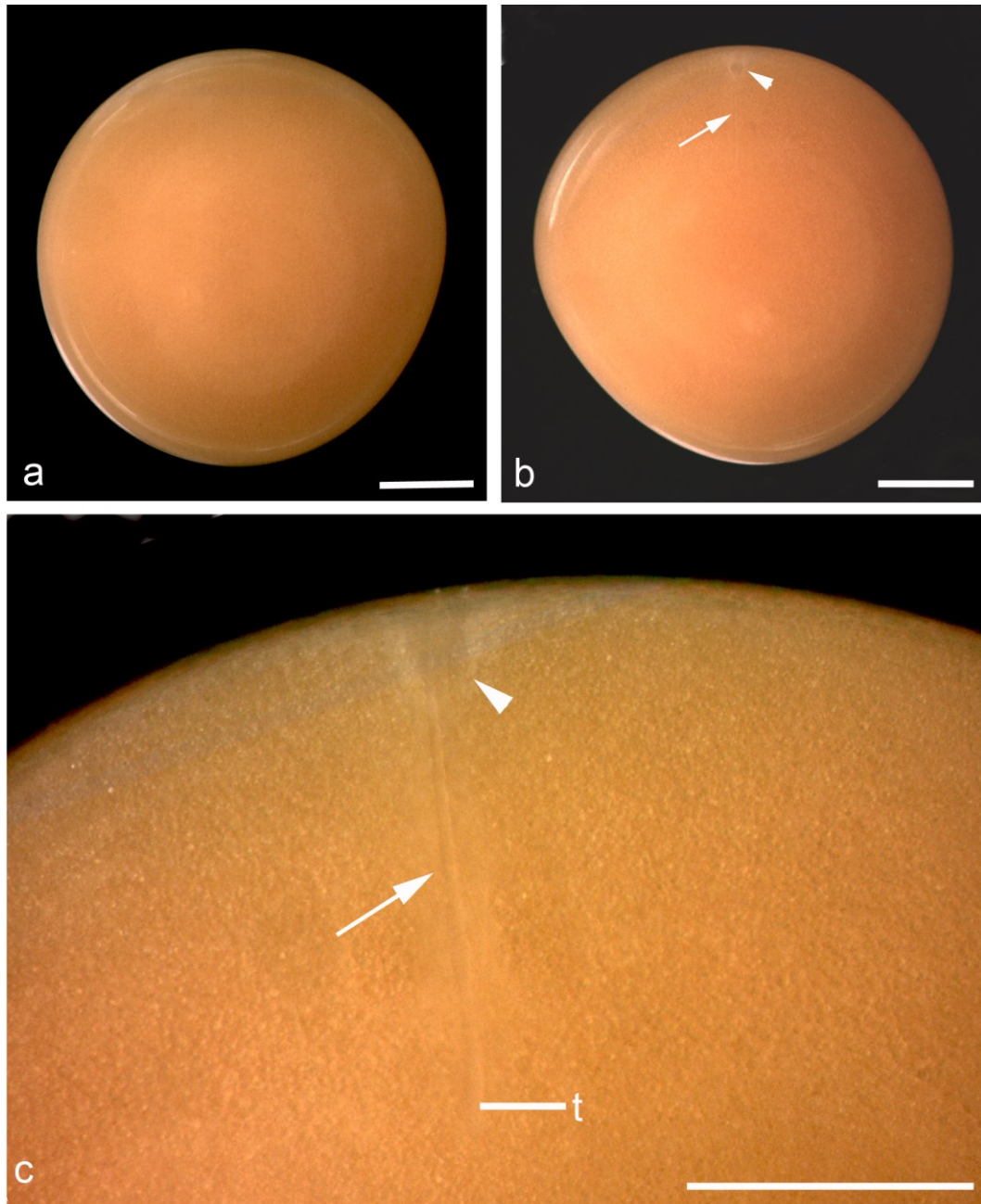


Fig. 52. First stages of the embryonic phase in *Osteoglossum bicirrhosum*. [a] Stage 1: freshly captured eggs, around 3 hours after released from male's mouth. [b] Stage 2: embryonic development with the axial trunk-tail (arrow) and anterior part of the body, future head located (arrowhead), 8 hours. [c] Depicting figure b with magnified 5x larger showing clearly embryonic development comprising anterior part (arrowhead), neural plate and the trunk-tail mound (t). Scale bar = 3 mm.

Stage 4: brain regionalisation

There were plenty of red speckled pigments on the yolk as a first sign of blood circulation. Head region enlarged due to further development of the brain region. The anterior part shows the future upper and lower jaw. The heart became visible and beat regularly. The evacuation zone in comparison to the previous stage has become much larger. This occurred 36 hours after releasing the egg from male's mouth (**Fig. 54a-b**).

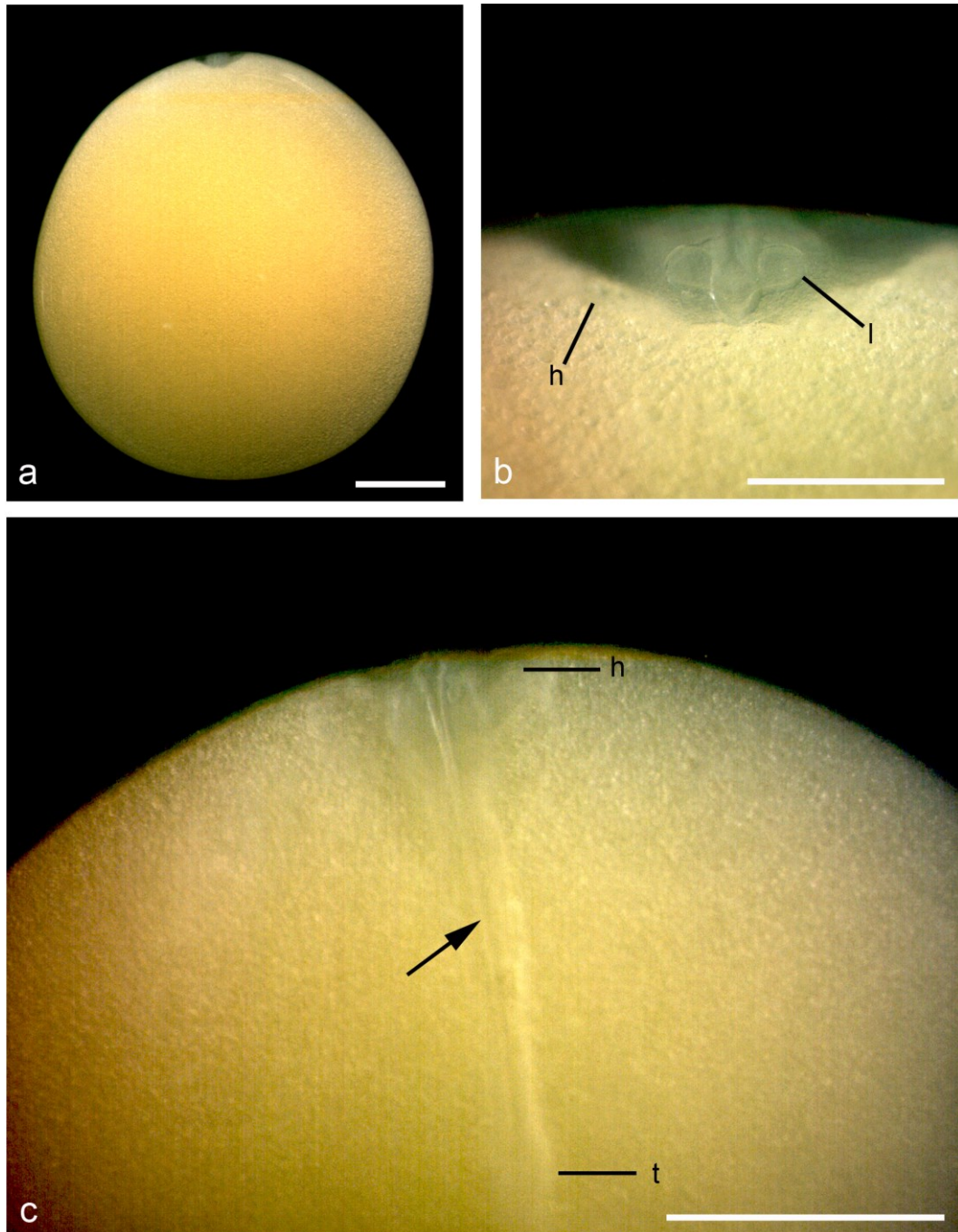


Fig. 53. Continuation of embryonic development in *Osteoglossum bicirrhosum*, stage 3 (a-c). [a] Around 21 hours after being released from male's mouth, view from front side. [b] View from front part of head region; note the beginning of heart and a pair of lens (l). [c] View from dorsal showing the neural plate (arrow). Scale bar = 3 mm.

Stage 5: early trunk-tail bud

At around 42 hours, a mass red blood cells and the forming heart can be seen inside the extended evacuation zone. The neural plate stretches rostrally and caudally now beyond the epidermal yolk-sac cover. Most of the caudal region of the neural anlage is now forming a rudimentary trunk-tail bud. **Figures 55 (a-d)** illustrate four different positions; embryo with 7 mm in total length still inside the egg envelope, thrashing

movements occurred as normal activity of the trunk and tail region. The eye lens and enlargement of the head can be seen here. Blood flow through the hepatic anlage that produced the hepatic-vitelline vein on the left anterior dorso-lateral surface of the yolk.

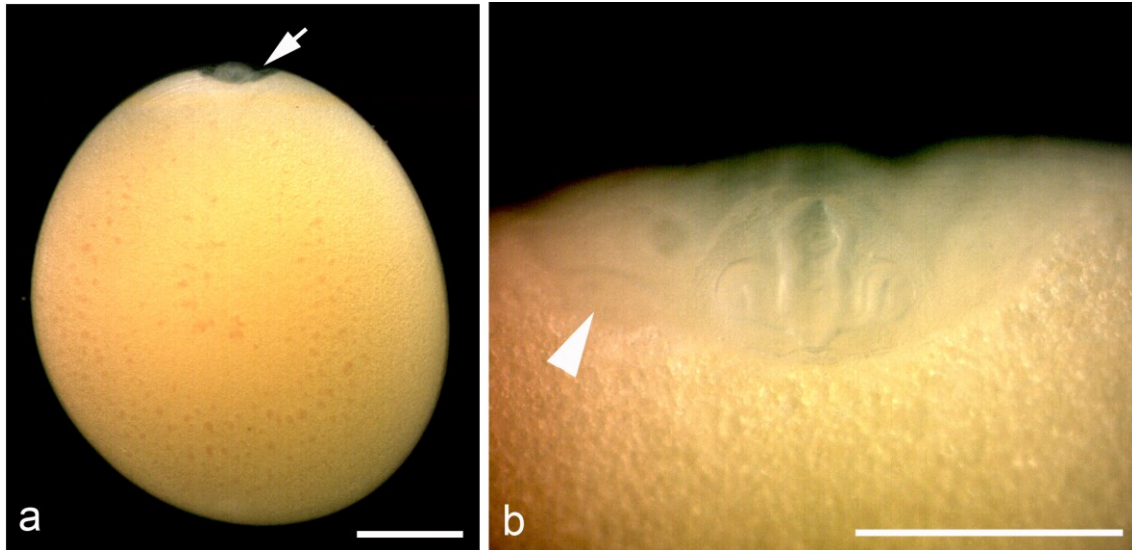


Fig. 54. Continuation of embryonic development in *Osteoglossum bicirrhosum*, stage 4. [a] The embryo 36 hours after release, showing massive red spots known capillaries on the entire yolk as first sign of blood circulation. [b] View from front part, showing enlarged head region. Note the heart beat, white arrowhead. Scale bar = 3 mm (a), 1 mm (b).

Stage 6: neural anlage

After 56 hours, the neural folds become contiguous along the dorsal midline of the trunk-tail, later on become fused to form a neural rod. Increased numbers of capillaries are seen on the entire yolk-sac and an opaque vena vittelina in direction to heart can be seen. If illumination is changed, contraction frequency is temporarily increased (**Fig. 56a-d**).

Stage 7: pectoral fin bud

The earliest somatic muscular activity occurs in the posterior trunk-tail region. Touching the embryo will first elicit a single twitch, then later a secondary backlash or even several thrashes of the tail. The neural plate projects rostrally and caudally now beyond the epidermal yolk-sac cover and is now formed like a club. A lateral symmetrical thickening of the neural folds suddenly emerged in the head region. At this stage the blood elements start circulating. The embryos are now 78 hours old and measure 10–12 mm in total length. Pectoral fins appear for the first time at this stage. Four branchial arches can be recognized behind the eye and above the heart. Only a thin layer of yolk separates the stomach from the ventral epidermal yolk sac cover (**Fig. 57 a-b**).

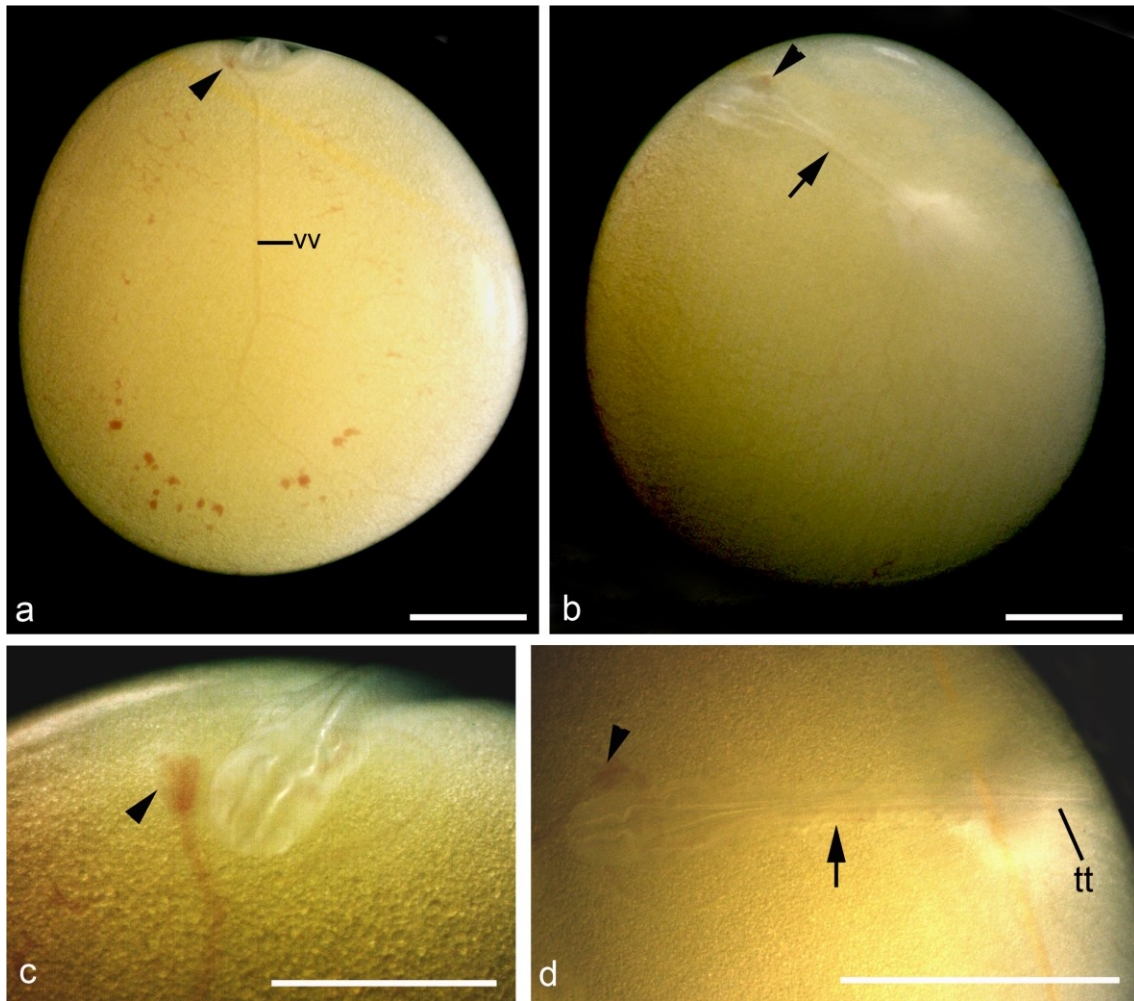


Fig. 55. Continuation of embryonic development in *Osteoglossum bicirrhosum*, Stage 5. [a] The embryo 42 hours after release, reduced number of red spots and first recognition of vena vitellina in direction to heart. [b] Left lateral view, showing the completely lengthened body with a lot of mesenchyme around the trunk tail. [c] Head region and the whole body [d] with trunk tail bud (tt) in larger magnification. Arrowhead shows the position of heart as orientation. Scale bar = 3 mm (a, b, d); 1 mm (c).

Stage 8: pre-hatching

The pre-hatching embryo appears with large black eye and gills at 160:00 (**Fig. 58a**). The pectoral fin bud becomes ellipsoid in shape, by dorsocaudal prolongation, and is now as big as the eye. The first formation of the rays in the caudal fin is now visible.

The muscular contractions of the embryo are such powerful actions that they affect the position of the entire body. These strong movements are indeed sufficient to change the orientation of the embryo inside the egg envelope. Later on, the embryo will start to break the egg envelope gradually with its tail which causes the egg envelope to lose its stability (**Fig. 58b**).

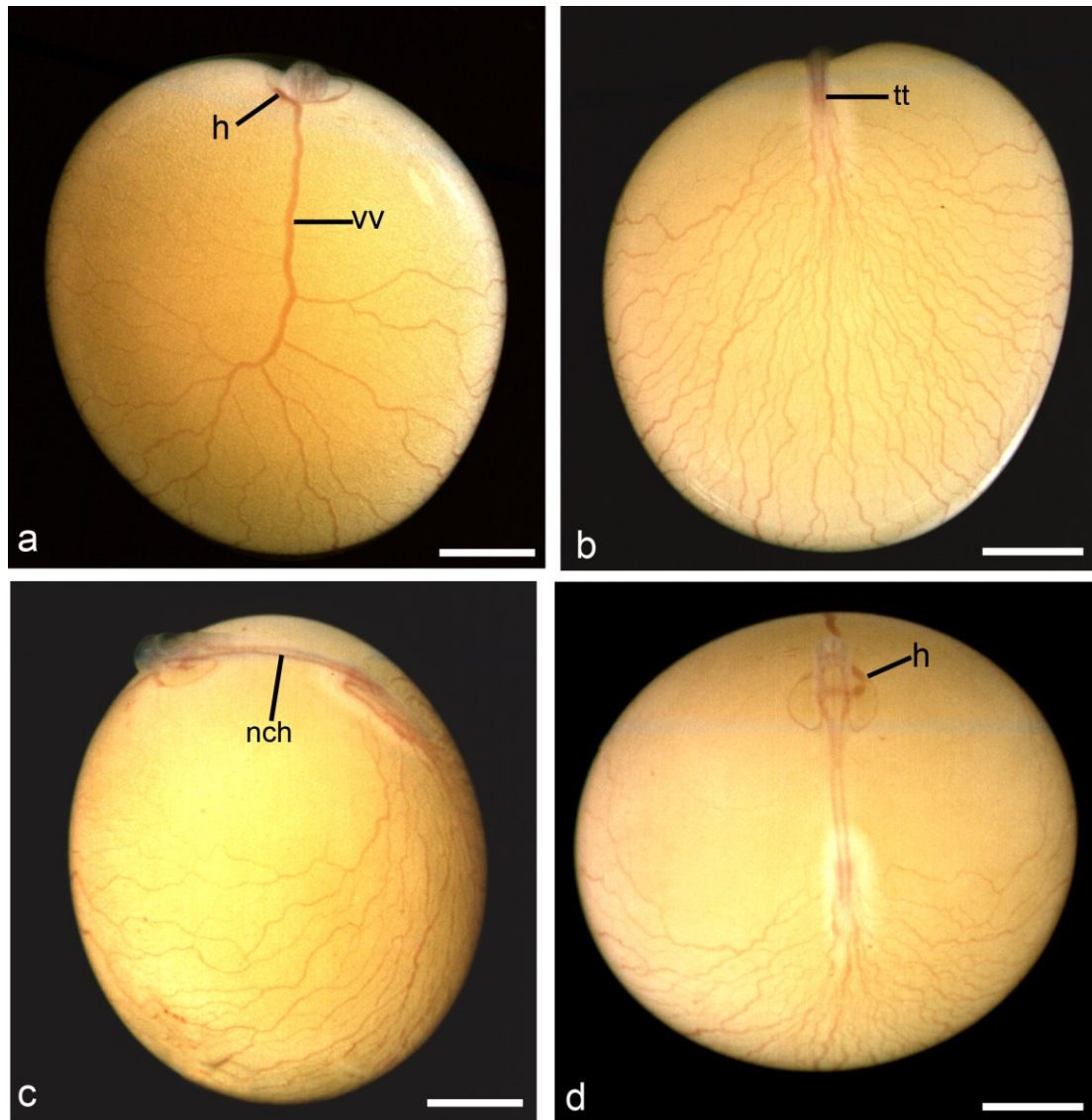


Fig. 56. Continuation of embryonic development in *Osteoglossum bicirrhosum*. [a-d] Stage 6: embryo in 56 hours after release in various positions, [a] front part, note the heart and the red vitelline vein (vv) in direction to the heart, [b] back part, note the trunk tail (tt) mound, [c] transversal, midline region of the neural plate with formed notochord (nch), and [d] view from dorsal, shows the entire body. Note active heart beat (h). Scale bar = 3 mm.

Stage 9: hatching

Hatching occurred at 162-166 h (around 7 days) after being released from parent's mouth. Shortly after rupture of the egg envelope, the free embryos often stay inside the egg envelope for a while (**Fig. 58c**). It took around 20 minutes releasing its complete body from the egg envelope. The mouth was not yet open but was completely formed and the heart is located on the ventral side of the branchial area. The yolk was covered with denser capillaries than previously. Now the embryos are undergoing a more complex blood circulation process and they measure around 16 mm (**Fig. 58d**).

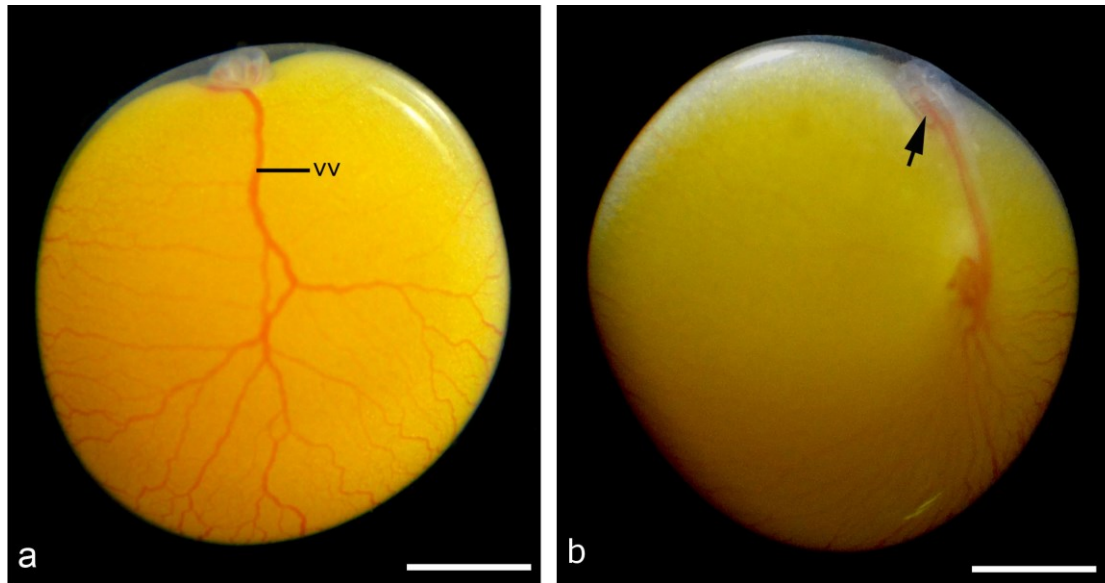


Fig. 57. Continuation of embryonic development of *Osteoglossum bicirrhosum*, Stage 7. [a] view from front part and [b] elongation of the trunk-tail and the emergence of pectoral fin bud (arrow), 72 hours. Scale bar = 3 mm

3.4.1.4.1.2 The eleutheroembryonic phase

The eleutheroembryonic will be characterized from stage 9 to stage 13, starting right after hatching until the embryos start to feed externally with the yolk sac still attached.

Stage 10: free embryo

The free embryos measure 17 mm on day 8 and stay instable with body on the upper part of yolk sac, though often also its bodies lie on the bottom of the tank. When disturbed, the free embryos wiggled their trunk and tail, but still cannot move the whole body with their large yolk sac. After release, embryo's motion was erratic though still moving forward. The pectoral fins were already capable of continuous movement and it was as big as the eye. The branchial arteries already formed five pairs of arches. An extensive band of melanophores appeared, covering the brain region. Embryos were either lying down on their dorsal side in a stable state or hanging with their large yolk sac unstably for a short time. The head structure is better defined (**Fig. 59a, day 8**).

Stage 11: jaw and branchial placodes

One to two days after hatching, the development of the circulatory system was obviously more extensive. The mouth was not completely developed and still couldnot be closed yet. Between head and yolk mass the first mesenchymal condensations of jaws and branchial arches could be recognized. The head process had already undergone a significant lift with several structures are being more defined. The branchial circulation was strongly developed and the gill filaments began to vascularise. The pharynx can be clearly seen through the transparent embryo. Its rostral edge attached to

the body, while the caudal edge sticks out from the body. The diameter of the yolk sac measured around 12-13 mm. The embryo now has 36 somites and measure 18 mm (**Fig 59b, day 9**). The alteration of head development from shortly after hatching at day 7 until day 9 is presented in **Figure 60** in larger magnification.

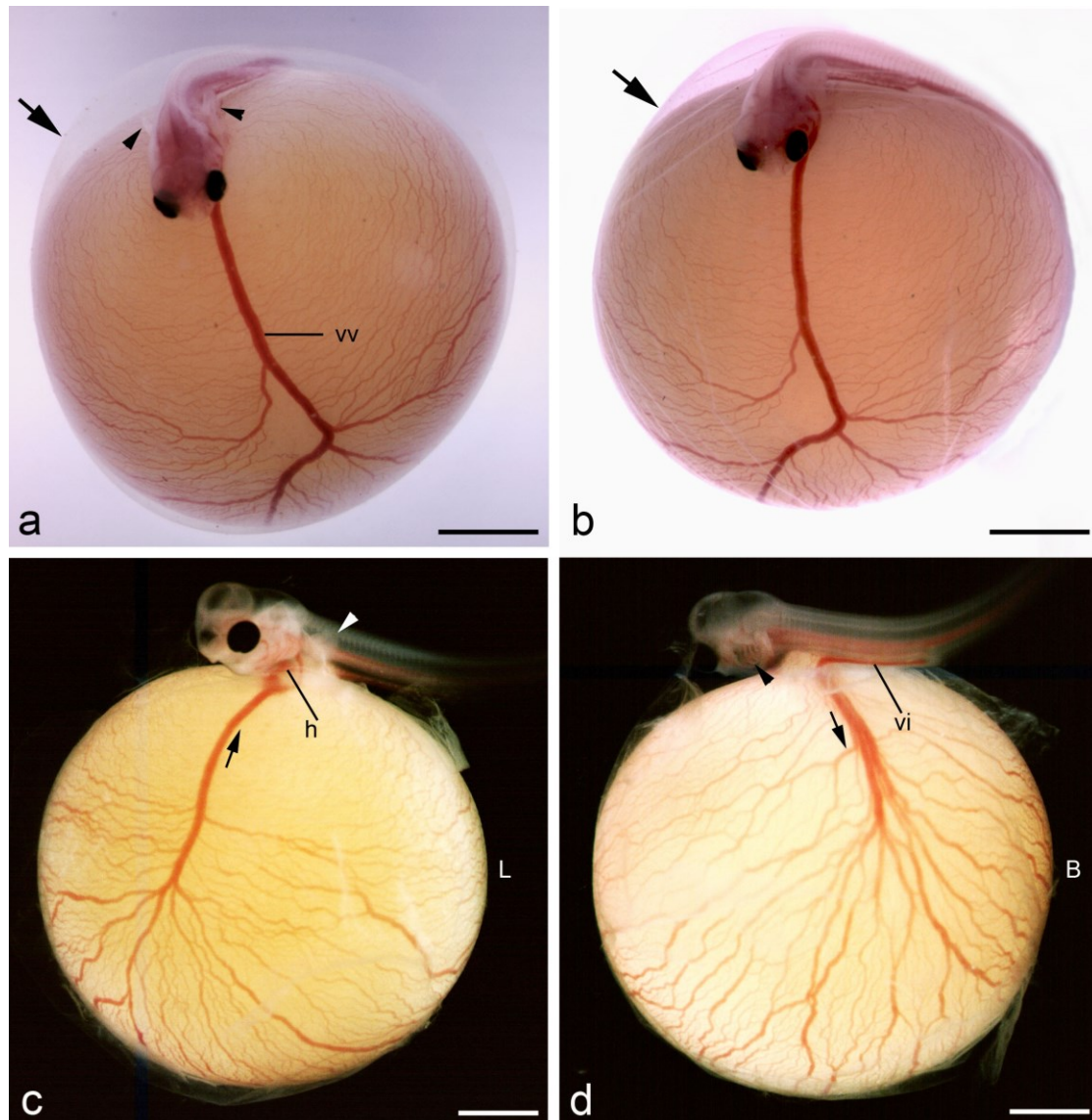


Fig. 58. Pre-hatching and free embryonic development of *Osteoglossum bicirrhosum*. [**a**] Stage 8: embryo with developed pectoral fin (black arrowhead). Note the strong red vena vitellina (vv). [**b**] Embryo started to break through the egg envelope by assertively moving its tail, 160 h. [**c**] Stage 9: Hatching, the free embryo is still attached to the remains of the egg envelope, view from left lateral (L). Note the pectoral fin bud (white arrowhead), 162-166 h. [**d**] View from behind (B), note the branchial arches (black arrowhead) and direction of blood circulation (black arrow). h – heart, vi – vena caudalis inferior. Scale bar = 4 mm.

Stage 12: initiation of barbels

The pelvic fin bud is already formed as a membranous bud, at the anterior to the anal fin and develops from this stage onward. Melanophores blacken at the edge of the lower

mouth; it's the sign of the barbels formation. The lower jaw has elongated and straightened. The mouth was entirely developed and moved rhythmically. The anlagen of the dorsal and anal fins appear simultaneously as denser concentrations of mesenchyme in the fin fold. The formation of the first rays in the caudal fin is distinct now. From this stage onward, the mobility has increased. The depicted individual measures 24 mm at this stage (**Fig. 61a, day 12**).

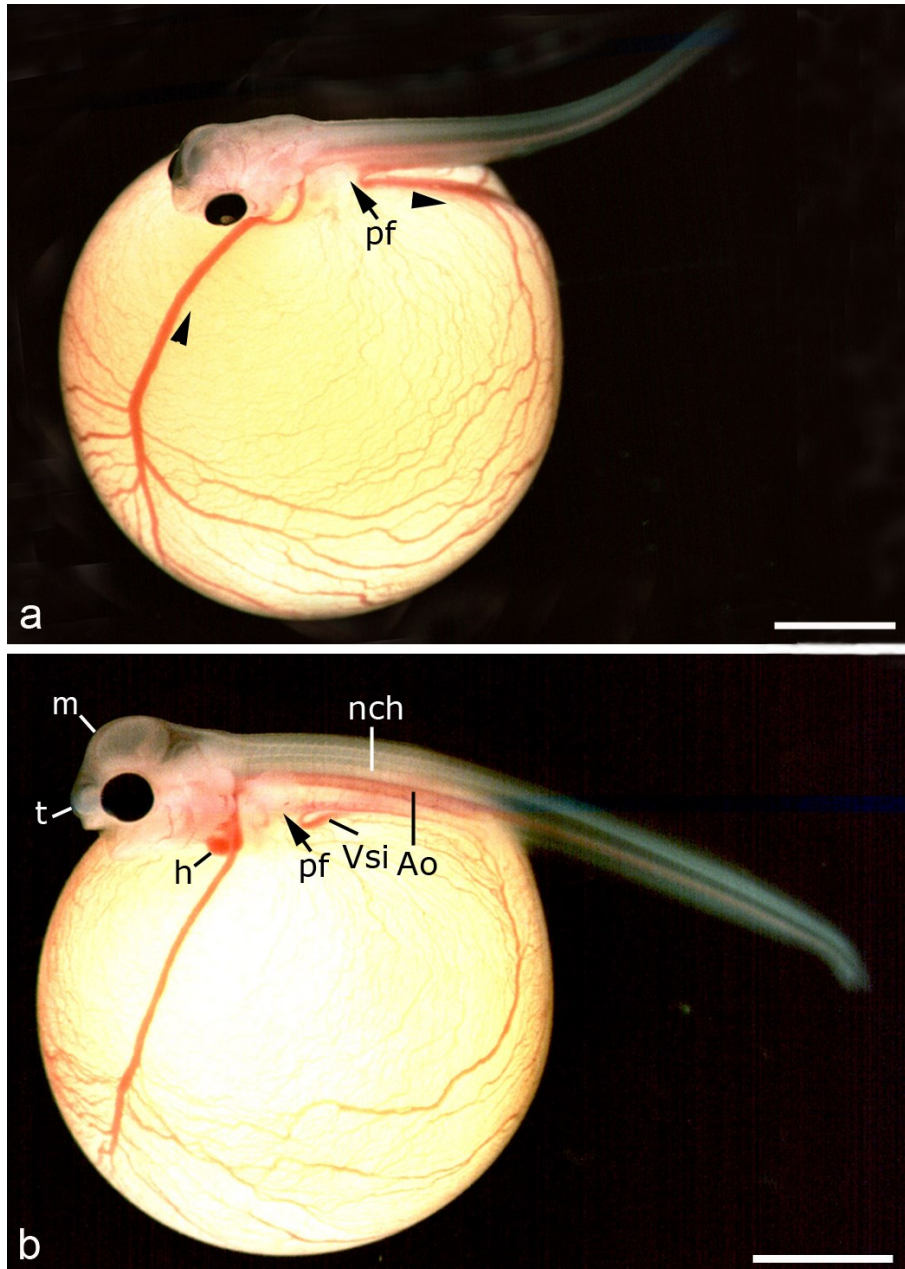


Fig. 59. Free embryonic development of *Osteoglossum bicirrhosum*. [a] Stage 10: Almost 15 hours after hatching with completed rupture of the egg envelope. Note the direction of blood circulation to the heart, day 8. [b] Stage 11: free embryo, 9 days. Note the ellipsoid shaped of pectoral fin (pf) with an open mouth. h – heart, er – eye ring, t – tela cephalon, m – mesencephalon, Vsi – vena subintestinalis, nch–notochord, Ao – aorta dorsalis. Scale bar = 3 mm.

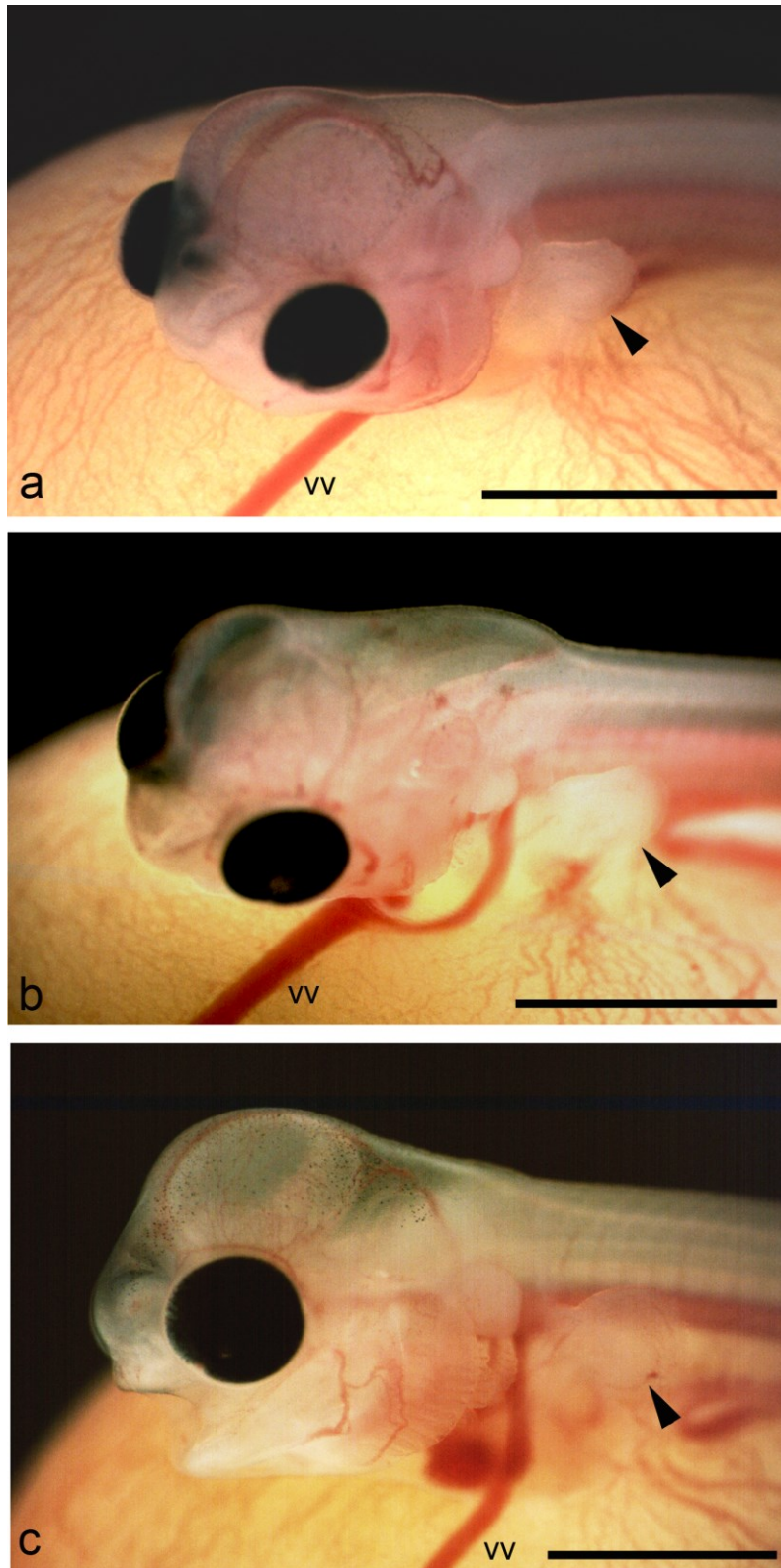


Fig. 60. Alteration of head structure of free embryo from stage 9 to stage 11 in *Osteoglossum bicirrhosum*, larger magnification. [**a**] Stage 9: the head is still bent downwards along the yolk sac and black pigmentation around the forebrain can be seen, day 7. [**b**] Stage 10: head elongation particularly on the posterior part, day 8. [**c**] Stage 11: head lifted with an open mouth, 9 days. Note the ellipsoid shaped of pectoral fin (pf). Scale bar = 2 mm.

Stage 13: ventral fin bud and swim bladder

At this stage the embryos are very actively swimming although only for a short distance. The swim bladder starts to elongate dorso-rostrally. At this stage, the pectoral fins are completely formed and functional with their 6 segmented lepidotrichial rays. Dorsal view of head region during this stage shows pectoral fins as two-equal wings. The head and around the dorsal part are densely covered with black pigments. The oval shaped yolk sac has gradually changed to spherical shape onward. The yolk was covered with more capillaries than previously. The surface of the still large yellow yolk sac was permeated by numerous finer capillaries than in the previous stage. Now the eleutheroembryos reached 28 mm in total length and the diameter of yolk sac measure 12 mm now (**Fig. 61b, day 15**). A large number of embryos in this stage are presented in **Figure 62** showing embryos lying unstably on the ground.

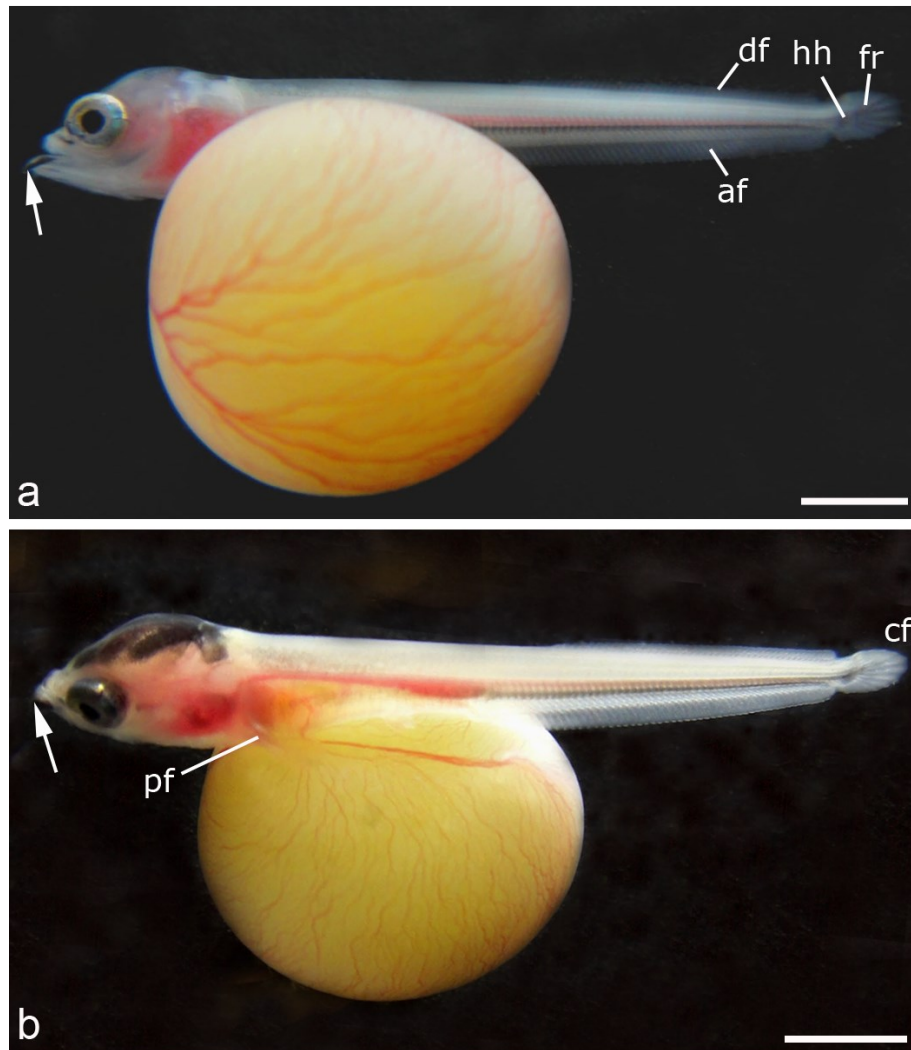


Fig. 61. Starting the eleutheroembryonic phase of *Osteoglossum bicirrhosum*. [**a**] Stage 12: massive head pigmentation, day 12 [**b**] Stage 13: developed fins, note the migrating of yolk into the abdominal cavity, day 15. Scale bar = 4 mm.

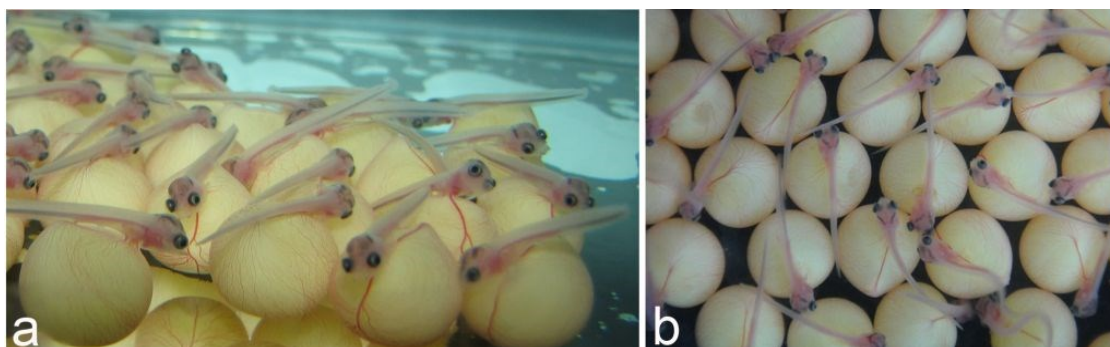


Fig. 62. A mass of free embryos of *Osteoglossum bicirrhosum* with age of 15 days, taken from the aquarium. [a, b] Embryos lie unstably on the bottom of the tank with little movement. Note the pigmentation around the head region and caudal part.

Stage 14: late eleutheroembryo

The barbels elongate in shape and measure 1 mm. Silver colour intensifies around the eye to the operculum region. A pair of ventral fins on the base part of the belly can be seen from this stage onward. It sticks to the yolk sac cavity. Two long, black-pigmented rows elongated dorso-rostrally and reached the anal fin. Melanophores covered almost all part of pectoral fins and left the outer part white, as well as around the area of operculum and the snout part. The late eleutheroembryos now measure 34 mm and the diameter of yolk sac still measure 12 mm (**Fig. 63a, day 24**).

3.4.1.4.2 The juvenile period

Stage 15: juvenile - 1 (Mixed feeding)

The pectoral fins develop with dense black pigments on the edge of the fins. Opaque melanophores covered the whole body with its particular aggregations at the top of the head and with very dark dark rows along the dorsal midline, along the intestine and the bases of the dorsal and anal fins. At this stage the juveniles start to feed exogenously concurrently with endogenous nutrient-utilization. The juveniles measure 38 mm (**Fig. 63b, day 26**).

Stage 16: juvenile - 2

The yolk sac has very much declined and the pelvic fin has developed further. Dense network melanophores covered the head region and also two dark rows along the dorsal midline and the centre part of the body to the caudal fin emerged. The barbels measure 2 mm and the juveniles measure 40 mm at this stage (**Fig. 63c, day 30**).

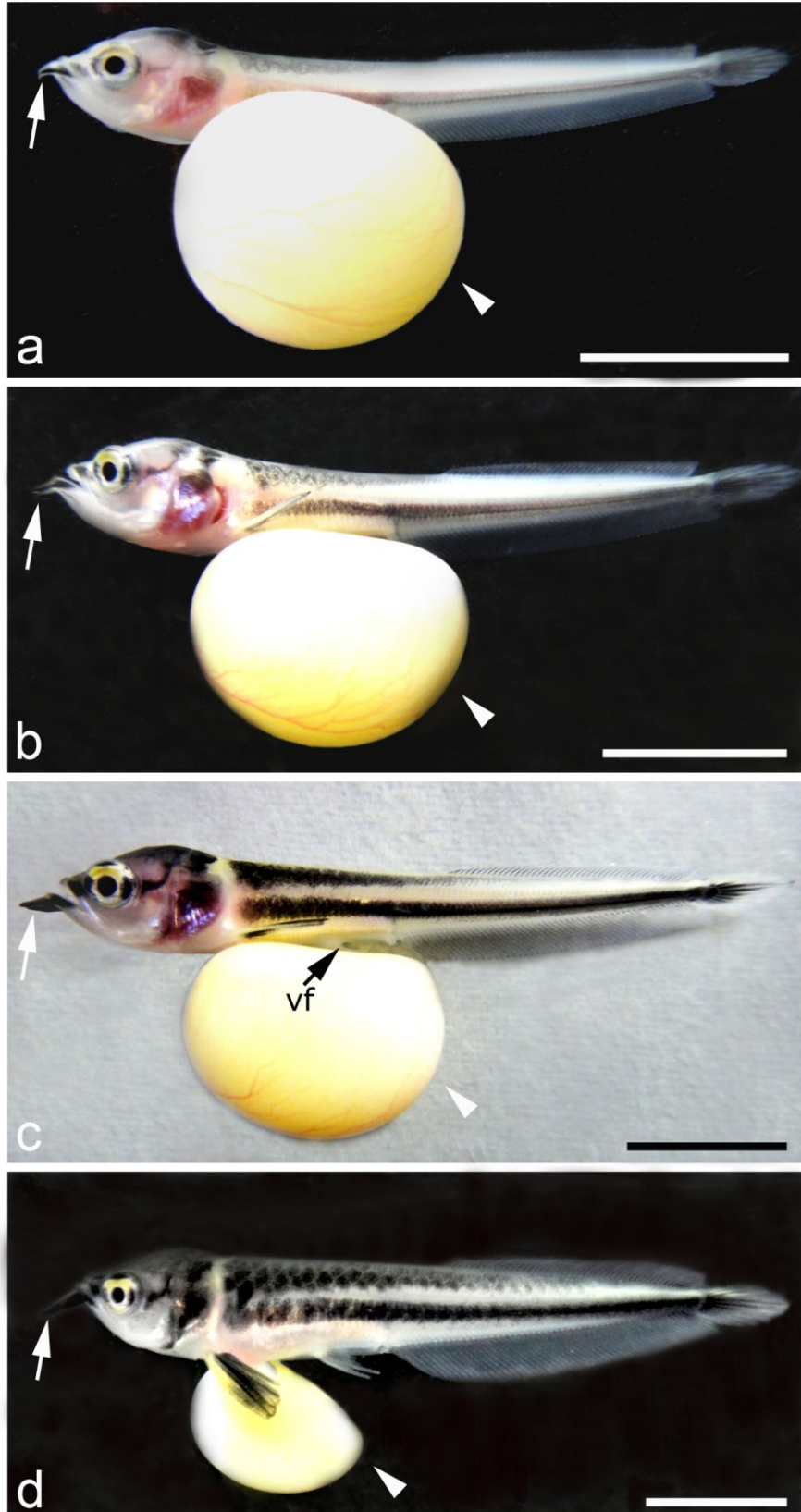


Fig. 63. [a] Continuation of the eleutheroembryonic phase and [b-d] starting point of the juvenile stage in *Osteoglossum bicirrhosum*. [a] Stage 14: late phase of the eleutheroembryo, day 24. [b] Stage 15: first juvenile, day 26. [c] Stage 16: a-30-day juvenile. [d] Stage 17: scales initiation, day 36. Scale bar = 10 cm.

Stage 17: juvenile - 3

The caudal part of yolk sac has released from the body due to the elongation of ventral fin. Usually the yolk mass is no longer rounded as previous stages, but has become a semicircle-shaped. Black pigmentation appears stronger along the body and the scales commence to develop from this stage onward. The mouth is able to close completely at this point. The pelvic fins are well-visible. The juveniles measure 45 mm at this stage (**Fig. 63d, day 36**).

Stage 18: juvenile - 4

The pelvic fins reached 8 mm in total length and the barbels are 3 mm long. The yolk sac has elongated quite a lot and has become tube-shaped. The depicted individual measures 48 mm (**Fig. 64a, day 40**).

Stage 19: juvenile - 5

The pelvic fins measure 9 mm and the barbels are still 3 mm long. The yolk sac is shorter than in the previous stage. The specimens now measure 56 mm (**Fig. 64b, day 50**).

Stage 20: juvenile - 6

Yolk sac capillaries somehow reduced although still clearly recognizable as to their origin. The barbels measure 4 mm and the pelvic fins measure 10 mm. The specimens measure 60 mm at this stage (**Fig. 64c, day 64**).

Stage 21: juvenile - 7

The barbels measure 5 mm and the pelvic fins reached 13 mm in length. The yolk almost disappeared within the body cavity walls, although a tiny rest of it remained. The juveniles measure 80 mm (**Fig. 64d, day 75**).

Stage 22: juvenile - 8

The barbels measure 6 mm now and the elongate pelvic fin has reached 15 mm in length at this stage. The body of the juvenile has elongated further and developed large silver scales. The linea lateralis shows a long line starting from behind the head region and somehow with stripe line showing a 135° shaped. The juveniles now measure 125 mm (**Fig. 64e, day 100**). A glance of all development stages in *Osteoglossum bicirrhosum* is presented in **Table 8** as below.

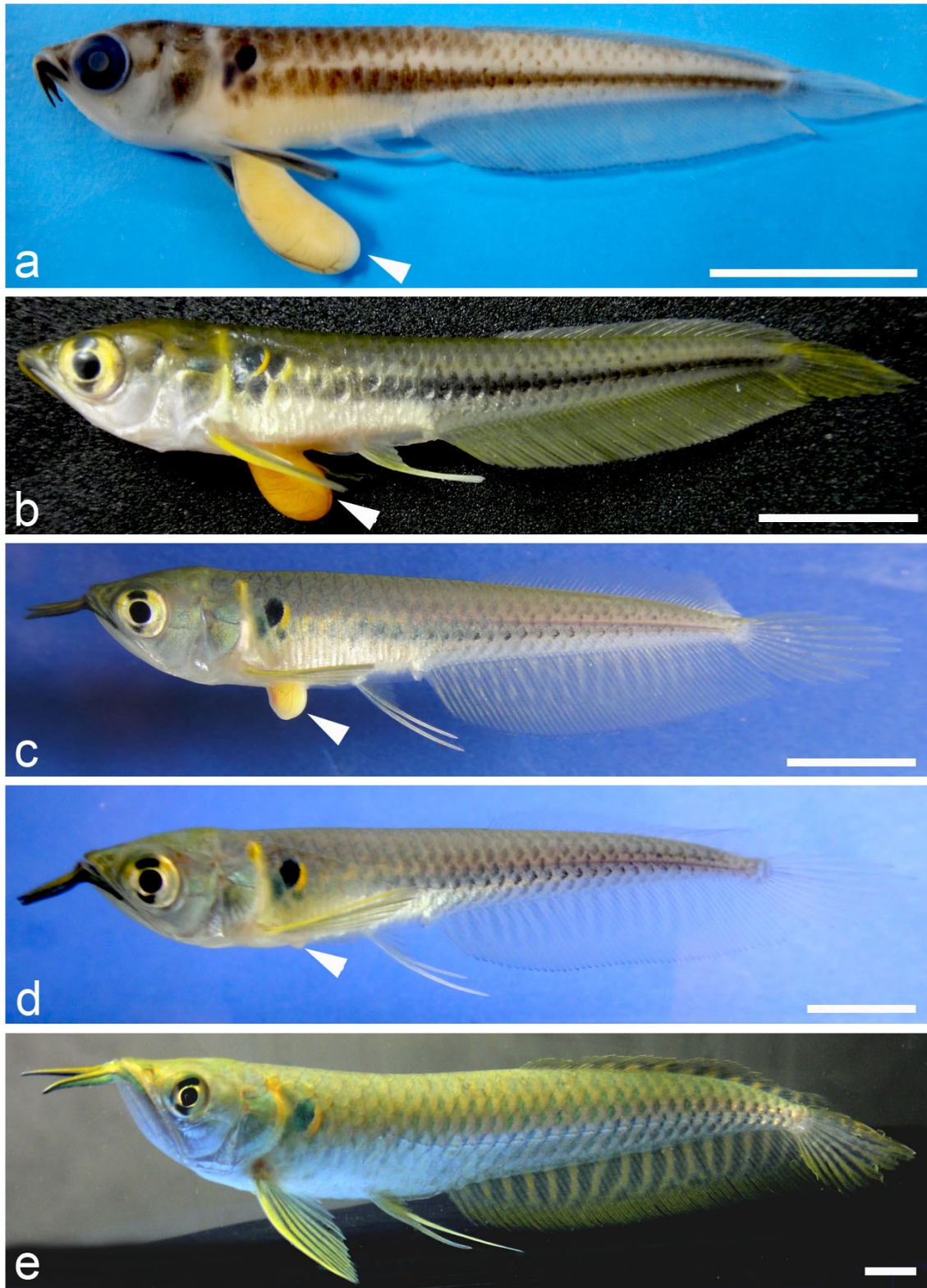


Fig. 64. Continuation of the juvenile sequences in *Osteoglossum bicirrhosum*. [a] Stage 18: a long tube-shaped yolk sac, day 50. [b] Stage 19: much reduced yolk sac. [c] Stage 20: tiny remnant of yolk sac, day 64. [d] Stage 21: juvenile prior fully absorbed yolk sac, day 75. [e] Stage 22: completely disappeared of yolk sac also called as late juvenile state, day 100. Scale bar = 10 mm.

Table 8. Overview of developmental stages in *Osteoglossum bicirrhosum* (28 °C), Determination after Balon (1975)

Period	Phase	Stage	Age	Characteristics	Figure
Embryonic	Embryonic		(hrs:min)		
		1	03:00	Freshly spawned egg	52a
		2	08:00	Neural plate	52b-c
		3	21:00	Onset somatogenesis	53a-c
		4	36:00	Brain regionalisation	54a-b
		5	42:00	Early trunk-tail	55a-d
	Eleutheroembryo	6	56:00	Neural anlage	56a-d
		7	78:00	Pectoral fin bud	57a-b
		8	160:00	Pre hatching	58a-b
		9	162:00–166:00	Hatching	58c-d
		10	D6–D7	Free embryo	59a
		11	D8	Jaw and branchial placodes	59b
Juvenile		12	D12	Initiation of barbels	61a
		13	D15	Ventral fin bud and swim bladder	61b
		14	D24	Late eleutheroembryo	61a
		15	D26	Juvenile 1	63b
		16	D30	Juvenile 2	63c
		17	D36	Juvenile 3	63d
		18	D40	Juvenile 4	64a
		19	D50	Juvenile 5	64b
		20	D64	Juvenile 6	64c
		21	D75	Juvenile 7	64d
		22	D100	Juvenile 8	64e

4 DISCUSSION

4.1 Overview of environmental triggers of gonad development

4.1.1 Family Mormyridae

The environmental factors (decreasing conductivity, constant water level and slight variation of temperature) applied in the experiment with one female and three males clearly showed a significant influence on gonad maturation in *Petrocephalus soudanensis*. The female has shown a largely swollen abdomen. This is in accordance with Kirschbaum (2006) and similar to other investigated mormyrids (Schugardt and Kirschbaum, 2004). The pair also established territories in the tank, while conductivity was lowered; however, no spawning occurred. This could be due to stress factors such as insufficient food during the breeding period or a too small breeding tank. Kirschbaum (1992) stated that stress factors prevent the completion of reproductive behaviour. The *P. soudanensis* used in this experiment had been used in Kirschbaum's experiments (2006) with successful breeding.

In the first breeding experiment with *Petrocephalus catostoma* (four males and female), a pair established its territory in the tank, however, no spawning was observed. Similar to the second breeding experiment with a female and two males, neither courtship behaviour nor spawning was observed. The influence of environmental factors decreasing conductivity, constant water level and slight variation of temperature on gonad development therefore remains unclear in this species. The breeding experiments failed in *P. catostoma* presumably due to the age of the specimens. Since the exact age was unknown, it is possible that they had already reached the senescent period. In fact, there was a significant decrease in total weight of the specimens within a 9-month experimental period.

4.1.2 Family Notopteridae

In the two breeding experiments with *Xenomystus nigri*, there is no clear influence of environmental factors (decreasing conductivity, constant water level and slight variation of temperature) on gonad maturation. In this study, two females showed gonad maturation in the breeding group I (9 females, 7 males). Three females also showed swollen bellies from the breeding group II comprising nine females and male. Thus, there was a mixed reaction since swollen bellies were found during rainy (decreasing conductivity) and dry season (increasing conductivity) in both experiments. No spawning activity occurred in both experiments. Courtship behaviour occurred sporadically in these two breeding groups. Apparently the small size of the breeding

tank and missing of a preferable spawning substrate could be the main reason for the failing of the breeding experiments. Nyonje (2006) stated that the three environmental factors imitation of rain, decrease conductivity and increase water level do not regulate gonad maturation in *X. nigri*.

In the dissected gonads of *X. nigri*, there were no ovulated eggs found, although some females' gonads contained oocytes in maturation stage (MC: 7.70 %; 7.63 % of breeding group I and MC: 8.3 % of breeding group II). The ovaries were filled with different stages of oocytes indicating fractional spawner.

In order to collect data for ontogeny development, trials of artificial reproduction have been applied to the selected pairs with swollen bellies. Intense courtship behaviour was still seen after the hormonal treatment, even though attempts of hormonal injection failed as no spawning occurred. In most cases of fractional spawners, artificial reproduction is difficult to perform.

Similar to *X. nigri*, there is no obvious reaction to the environmental factors (decreasing conductivity, constant water level and slight variation of temperature) in *Notopterus notopterus*. A female of the breeding experiment I (two females and male), indeed showed a swollen belly after the onset of decreasing conductivity. Intense courtship behaviour observed until the first spawning took place after six months experimental period. In the breeding group II comprising a pair of *N. notopterus*, swollen bellies as indication for gonad maturation and courtship behaviour were often seen at high levels of conductivity. The influence of environmental factors on the gonad maturation and courtship behaviour in *N. notopterus* are therefore unclear. Apparently endogenous factors play an important role predominantly in regulating gonad maturation and in provoking courtship behaviour in this species. A study of Weitkamp (2005) on *N. notopterus* revealed that none of the environmental factors tested (i.e conductivity, temperature and water level) had significant influence on gonad maturation.

4.1.3 Family Pantodontidae

Two experiments were conducted in *Pantodon buchholzi* regarding environmental factors: constant water level, decreasing conductivity and slight variation of temperature. In the breeding group I containing seven males and five females, no spawning occurred even though a pair showed clear signs of courtship behaviour for several days during decreasing conductivity. In the breeding group II with one male and two females, neither gonad maturation nor courtship behaviour was observed during the

experimental period. This could be due to wrong feeding or maybe the applied parameters were not appropriate to induce the gonad maturation in this species.

Conductivity was drastically decreased a few times in the three additional breeding experiments with *P. buchholzi*, comprising breeding group combination (BGC) I (1♀, 1♂), BGC II (5♀, 4♂), and BGC III (4♀, 5♂). Neither a swollen belly nor courtship behavior was observed throughout these trials. It seems that environmental factors do not regulate gonad maturation in *P. buchholzi*. Britz (2004) reported that after several drastic water changes gonad maturation in *P. buchholzi* could be induced. Apparently maturation is controlled endogenously and the environmental factors only provoke ovulation in *P. buchholzi*, as found in *Polypterus senegalus* (Kirschbaum, 1992; 1995b; Yanwirsal, 2007).

4.1.4 Family Osteoglossidae

Based on the results of fieldwork in Colombia, while collecting the samples, this has shown that the rainy season (January to May) is the main spawning season in *Osteoglossum bicirrhosum*. This is confirmed by the experience of the local farmers that in general the onset of rainy season is often used as a definite sign to indicate the spawning season in *O. bicirrhosum*. This is in accordance with Argumedo (2005): breeding season occurs during the rainy season (in late November to early of July). In contrast to other osteoglossids, spawning mainly occurs prior to the onset of the rainy season in *Scleropages formosus* (Scott and Fuller, 1976; Suleiman, 2003).

4.2 Modes of reproduction in *Notopterus notopterus* and *Osteoglossum bicirrhosum* with comparison to other osteoglossomorphs

4.2.1 Reproductive guilds

This study confirms that *Notopterus notopterus* is a substrate spawner with a semi-transparent yellowish egg envelope. This is in accordance with Mookerjee and Mazumdar (1946), Ong (1965), Axelrod and Burgess (1981), and Pinxteren (1974). Substrate spawning is also performed by *Chitala chitala* (Pinxteren, 1974; Axelrod and Burgess, 1981).

This study also verifies that *Osteoglossum bicirrhosum* is a mouth breeder (Ungar, 1993; Argumedo, 2005) with a large (Wolfsheimer, 1964) translucent envelope. All the representatives of the genera *Scleropages* are mouth breeders (Merrick and Green, 1982; Lake and Midgley, 1970; Azuma, 1992; Brown, 1995). Nest building behaviour is seen in male mormyrids of *Pollimyrus isidori* and *Pollimyrus adspersus* (Diedhiou et al., 2007a); and in the two osteoglossids *Heterotis niloticus*

(Budgett, 1901a; Svensson, 1933; Johnels, 1954; Daget, 1957) and *Arapaima gigas* (Fontanele, 1948, 1952; Lüling, 1964, 1969; Neves, 1998). Budgett (1901a) and Svensson (1933) reported that only the species *Gymnarchus niloticus* built large floating nest.

4.2.2 Spawning time

Spawning in *N. notopterus* occurs mostly during day time. Friesse (1980) also reported that spawning in *N. notopterus* tends to occur in the early morning. In contrast, Smith (1933) and Pinxteren (1974) stated that spawning in *N. notopterus* occurs mainly at night. It seems that spawning in *N. notopterus* may occur at any time. In the Silver Arowana, *O. bicirrhosum* spawning takes place at night and in the early morning.

Spawning in *S. leichardtii* apparently takes place at night (Merrick and Schmida, 1984), similar to *Pollimyrus isidori* (Schugardt and Kirschbaum, 2006; Diedhiou et al., 2007b), *Mormyrus rume probocirostris* (Schugardt and Kirschbaum, 2004) and *Campylomormyrus cassaicus* (Schugardt and Kirschbaum, 2002), *Pantodon buchholzi* (Britz, 2004) and *Petrocephalus soudanensis* (Kirschbaum, 2006).

4.2.3 Left gonad

This study verifies that *O. bicirrhosum* and *N. notopterus* possess a single gonad located on the left side of the body, as observed also in *Xenomystus nigri* and *Osteoglossum ferreirai*. Therefore it confirms the statement of Nyonje (2006) and Argumedo (2005, 2009) concerning the presence of one gonad in *X. nigri*, *O. bicirrhosum* and *O. ferreirai*. A single gonad is also present in *Scleropages leichardtii* (Merrick and Schmida, 1984; Merrick et al., 1983; Lake and Midgley, 1970), *Heterotis niloticus* (Moreau, 1974), *Arapaima gigas* (Fontanele, 1948; Lüling, 1964), *Chitala ornata* (Smith, 1933) and in three mormyrids *Hyperopisus bebe* and *Mormyrus kannume* (Nawar, 1959; Scott, 1973) and also in *Hippopotamyrus pictus* (Blake, 1977). In addition, this study also confirms that *Pantodon buchholzi* possesses both ovaries. This is in accordance with Nysten (1962). The presence of both ovaries was also found in the Mooneye *Hiodon tergisus* (Glenn and Williams, 1976). Britz (2004) concluded that species with paired ovaries possess the plesiomorphic and those with only one ovary the apomorphic character.

4.2.4 Fractional spawner

The ovary of *O. bicirrhosum* and *N. notopterus* is filled with various stages of oocytes comprising stage I (primary growth) to stage IV (maturation stage) (based on Wallace and Selman, 1981). This indicates that both species are fractional spawners. Azadi et al.

(1995) showed that the ovary of *N. notopterus* was filled with three different stages (I to III) of oocytes. Similar to what Weitkamp (2005) found, the ovary of *N. notopterus* contained different stages of gonad maturation. Fractional spawning was also observed in *Pollimyrus isidori* (Kirschbaum, 1987; Kirschbaum and Schugardt, 1995); in *Mormyrus rume probocirostris* (Schugardt and Kirschbaum, 2004); in fishes of the *Paramormyrops magnostipes*-complex (Nguyen, 2011); and in *Xenomystus nigri* (this study; Nyonje, 2006).

4.2.5 Parental care

This study proved that *N. notopterus* shows parental care performed by the male as reported by other authors (Pinxteren, 1974; Smith, 1933; Axelrod and Burgess, 1981; Friese, 1980). Both *Chitala ornata* and *Chitala chitala* take parental care of their adhesive eggs. The male guards the eggs attached to different substrates (Smith, 1933; Southwell and Prashad, 1919). Trittelvitz (1986) and Sieraad (1999) assumed that there was no parental care in *Xenomystus nigri*.

In mormyrids, *Pollimyrus isidori* and *P. adspersus* are known to show behaviour of parental care (Kirschbaum and Schugardt, 2002; Diedhiou et al., 2007a), as well as in *Stomatorhinus* (Heymer and Harder, 1975). No signs of parental care was reported neither for *Campylomormyrus cassaicus* nor *Hippopotamyrus pictus* (Kirschbaum and Schugardt, 2002); nor for *Paramormyrops magnostipes*-complex (Nguyen, 2011). *Pantodon buchholzi* also does not perform parental care (Britz, 2004; Siegl, 1914; Schreitmüller, 1936; Vriends, 1978)

The male of *O. bicirrhosum* carries the eggs, embryos and juveniles for about two months in its mouth. According to several authors (Argumedo, 2005; Merrick and Green, 1982; Azuma, 1992; Brown, 1995; Takeshita, 1973; Scott and Fuller, 1999; Dawes et al., 1999) mouth breeding in the family Osteoglossidae: *O. bicirrhosum*, *S. leichardtii*, *S. jardini* is performed by the male. A recent paper by Schaefer (2010) reported that female of *Scleropages* was occasionally found carrying eggs, though this statement was denied by the breeders.

4.2.6 Egg adhesiveness

The adhesive eggs from *N. notopterus* were always attached to a substrat, i.e. either to a rock, a huge/flat stone or to a filter. This is in line with Srivastava et al. (2012), Friese (1980), Axelrod and Burgess (1981), and Pinxteren (1972). Adhesive eggs are also found in other notopterid species such as *Chitala ornata* (Smith, 1933) and in five mormyrids: *Hippopotamyrus pictus*, *Campylomormyrus cassaicus*, *C. phantasticus*, and *Mormyrus rume probocirostris* (Kirschbaum and Schugardt, 2002) and in the fishes of

Paramormyrops magnostipes-complex (Nguyen, 2011). Daget (1957) also reported that the eggs of *Heterotis niloticus* sink to the bottom of the nest, where they adhere to each other, forming a large mass of eggs.

This study reveals for the first time that the eggs of *O. bicirrhosum* are non-adhesive. Non-adhesive eggs can also be found in other osteoglossomorphs, such as *Pollimyrus isidori* and *P. adspersus* (Diedhiou et al., 2007a). Concerning *Pantodon buchholzi* (Britz, 2004) it is reported to produce buoyant eggs with some large oil globules. The eggs of *Hiodon alosoides* are semi pelagic eggs due to its single large oil globule (Battle and Sprules, 1960). Wallus (1990) reported that the eggs of *H. tergisus* and *H. alosoides* are non-adhesive and buoyant/semi buoyant.

4.2.7 Egg numbers per spawning

In this study the number of eggs per spawning in *Notopterus notopterus* varies from 15-225 eggs. Some previous authors reported number of eggs per spawning in between, namely 50 eggs (Ong, 1965), up to 90 eggs (Pinxteren, 1974) and around 30–100 eggs (Axelrod and Burgess, 1981). A higher number of eggs compared to *N. notopterus* was found in *Chitala chitala*, 300-500 eggs (Southwell and Prashad, 1919) and up to 3500 in *C. ornata* (Smith, 1933). In *Xenomystus nigri*, the number of eggs per spawning varies from 40–60 (Trittelvitz, 1986).

The male *Osteoglossum bicirrhosum* may carry around 23-220 eggs. This study presents higher numbers of eggs per spawning in *O. bicirrhosum* in comparison to Maupin (1967), Ungar (1993) and Wolfsheimer (1964), who reported that *O. bicirrhosum* spawned between 40, 135 and 150 eggs. Less number of eggs per spawning was also found in other osteoglossids, around 30–80 in *Scleropages formosus* (Azuma, 1992), around 30–110 in *S. leichardtii* (Allen et al., 2002), and 11.000 eggs in *Arapaima gigas* (Neves, 1998).

There is a wide variety of the quantity of eggs per spawning in other osteoglossomorphs. Regarding mormyrid species, *Pollimyrus isidori* lays 30–200 eggs (Kirschbaum, 1987; Kirschbaum and Schugardt, 1995); *P. adspersus* around 28–215 (Diedhiou et al., 2007a); *Petrocephalus soudanensis* 180–283; *C. phantasticus* about 250–400; *Mormyrus rume probocirostris* 128–728, and *Hippopotamyrus pictus* lays circa 66–772; *Campylomormyrus cassaicus* 121–1662 (Kirschbaum and Schugardt, 2002); and about 1000 eggs in *Gymnarchus niloticus* (Budgett, 1901a; Svensson, 1933).

4.2.8 Egg size

In this study, the size of freshly collected eggs from *Osteoglossum bicirrhosum* mainly measured 12 mm. This differs from the two previous authors Wolfsheimer (1964) and Aragao (1981) showed that the eggs of *O. bicirrhosum* are 16 mm and 13 mm large. In other osteoglossids, such as *Scleropages formosus*, the newly spawned eggs measured 19 mm in diameter (Shigeru et al., 1999), around 15–18 mm (Azuma, 1992), reached up to 10 mm in *S. leichardtii* (Lake and Midgley, 1970), and 3 mm (Menezes, 1951) in *Arapaima gigas*. The egg size varies from 2.5 mm (Budgett, 1901a) and circa 2.5-2.8 mm (Daget, 1957) in *Heterotis niloticus*; 2.8 mm (Fontanele, 1952), and 4.2 mm (Fontanele, 1948).

The newly spawned eggs of *N. notopterus* in this study were 3.8–4 mm in diameter. This is in accordance with Axelrod and Burgess (1981), Svensson (1933), and a recent study of Srivastava et al. (2012). In contrast to what Friese (1980) and Azadi et al. (1995) reported, the eggs of *N. notopterus* are 3.5 mm and 3.10 mm in size.

Except for the eggs of *Gymnarchus niloticus* which measure 10 mm in diameter (Budgett, 1901b), it seems that the size of the eggs in notopterids and osteoglossids are larger than in other osteoglossomorphs. As seen in the egg size of mormyrids such as 1.8 mm in *Hyperopisus bebe* (Johnels, 1954) and *Petrocephalus soudanensis* (Kirschbaum, 2006); 2 mm in *Paramormyrops magnostipes*-complex (Nguyen, 2011), *Pollimyrus isidori* (Kirschbaum, 1987; Diedhiou et al., 2007), *Marcusenius mento* (Boulenger, 1890; Schugardt and Kirschbaum, 2006), *Campylomormyrus tamandua* (Günther, 1864), and *Campylomormyrus cassaicus* (Schugardt and Kirschbaum, 1998); 3 mm in *Xenomystus nigri* (Trittelvitz, 1986), *Campylomormyrus phantasticus* and *Hippotamyrus pictus* (Kirschbaum and Schugardt, 2002).

Concerning *Pantodon buchholzi* the egg size measure 2 mm (Mohn, 1976a), circa 2.2-2.4 mm (Britz, 2004). In hiodotid species, egg of *Hiodon tergisus* and *H. alosoides* measures 4 mm and 3.3-3.9 mm in diameter (Wallus, 1990).

4.2.9 Hatching

In this study it has been shown that *N. notopterus* hatched at 7–8 days after spawning at a of temperature of 27 °C This is a bit different to what other authors found: 5–6 days after spawning at 26-28 °C (Srivastava et al., 2012); 5–6 days after spawning (Pinxteren, 1974); after 7 days of spawning (Axelrod and Burgess, 1981); in 6 days after spawning at 28 °C (Friese, 1980). In *Chitala chitala* hatching occurred around five days after spawning (Hossain, 1999; Radheyshyam and Sarangi, 2005).

Hatching in *Osteoglossum bicirrhosum* occurs around 7 days at 28 °C after being released from the male's mouth. In other osteoglossids, hatching occurs in 7-14 days in *Scleropages leichardtii* (Lake, 1971; 1978), 12 days after spawning in *S. formosus* (Azuma, 1992), five days (Neves, 1998) and around 10–14 days in *Arapaima gigas* (Lake and Midgley, 1970).

In *Heterotis niloticus* (Budgett, 1901a) hatching occurred around two days after spawning; after three days in fishes of the *Paramormyrops magnostipes*-complex (Nguyen, 2011), *Petrocephalus soudanensis* (Kirschbaum, 2006; Kirschbaum and Schugardt, 2002), *Pollimyrus isidori* (Kirschbaum, 1987; Kirschbaum and Schugardt, 1995; Diedhiou et al., 2007) and *Mormyrus rume probocirostris* (Kirschbaum and Schugardt, 1995); and after three days (Britz, 2004; Schreitmüller, 1936) and 4–6 days in *Pantodon buchholzi* (Siegl, 1914). In *Hiodon alosoides* hatching occurs about 14 days after spawning (Wallus, 1990).

However the developmental stage at hatching is influenced by environmental factor such as temperature and oxygen conditions (Hamor and Garside 1979; Peñáz et al. 1983; Heming, 1982).

4.2.10 Size of free embryos at hatching

In this study, free embryos of *N. notopterus*, directly after hatching, measured 10.5 mm in total length, which however, is slightly longer to what Axelrod and Burgess (1981) found, according to whom newly hatched *N. notopterus* measured 7 mm. Newly hatched embryos of *N. notopterus* measured 8.3 mm in total length (Mookerjee and Mazumdar, 1946) and 8.0 ± 0.5 mm long (Srivastava et al., 2012). In other notopterids, free embryos of *Chitala chitala* measured 13.8 mm (Southwell and Prashad, 1919) and 12 mm in *C. ornata* (Smith, 1933).

The size of the free embryos in *Osteoglossum bicirrhosum* in this study measured 16 mm in total length at hatching which occurred 7 days after being released from the mouth of male's parent. Argumedo (2005) reported that the free embryos of *O. bicirrhosum* are 17-20 mm long after hatching.

S. leichardtii's large-sized embryos measured 36 mm (Lake and Midgley, 1970) and 15 mm at hatching (Merrick and Green, 1982). Free embryos in *Heterotis niloticus* measured in total length of 7.5 mm (Daget, 1957), whereas 11.6 mm long in *Arapaima gigas* (Neves, 1998; Fontanele, 1948, 1952);

A variable hatching size of embryos (in total length) was also reported in other osteoglossomorphs: 3.4 mm in *Pollimyrus isidori* (Diedhiou et al., 2007b) and

P. adspersus (Diedhiou et al., 2007a); 3.5–4 mm in the fishes of *Paramormyrops magnostipes*-complex (Nguyen, 2011) and 4.2 mm in *Campylomormyrus cassaicus* (Schugardt and Kirschbaum, 1998), 7 mm in *Hippopotamyrus pictus* (Kirschbaum and Schugardt, 2002); around 7.2 mm in *Petrocephalus soudanensis* (estimated from Fig. 3 in Kirschbaum, 2006); 4.2–4.6 mm in *Pantodon buchholzi* at 29 °C (Britz, 2004); ca. 7 mm in *Hiodon tergisus* (Wallus, 1990) and 7.2–7.6 mm in *H. alosoides* (Battle and Sprules, 1960).

4.2.11 Onset of exogenous feeding

Exogenous feeding was observed for the first time in *N. notopterus* larva after 17 days with 16.2 mm in total length. Similar to *Chitala ornata*, exogenous feeding started once larvae were 13–18 mm long and 16–17 days old (Smith, 1933). The resorption of yolk sac in *N. notopterus*, observed around 18 days after spawning with 15.3 mm in total length (Mookerjee and Mazumdar, 1946) and at the age of 22 days (Axelrod and Burgess, 1981), can be categorized as the onset of exogenous feeding. *O. bicirrhosum* started mixed feeding on the 26th day with 38.2 mm in total length. Shigeru et al. (1999) reported that 64 days after spawning the fry of *S. formosus* began to leave the male's mouth, but only for a short time, and after 111 days with a total length of 62 mm it proceeded to do so permanently. 60 days after spawning the fry for the first time swam free for a longer period of time, though they still stayed close to the male's mouth (Azuma, 1992).

Regarding other osteoglossomorphs, reports of the emergence of exogenous feeding are: 3 days (Kirschbaum and Schugardt, 2002) and 4 days (Kirschbaum, 1994; Diedhiou et al., 2007) in *Pollimyrus isidori*; 8 days after hatching in *Mormyrus rume probocirostris* (Kirschbaum and Schugardt, 1995), *Campylomormyrus cassaicus* (Schugardt and Kirschbaum, 1998), *Hippopotamyrus pictus* (Kirschbaum and Schugardt, 2002), *Petrocephalus soudanensis* (Kirschbaum and Schugardt, 2002); in the fishes of *Paramormyrops magnostipes*-complex (Nguyen, 2011), and 4–5 days in *Pantodon buchholzi* (Britz, 2004).

The onset of exogenous feeding is depending on the egg size of the species. It seems that the larger the egg size, the later the onset of exogenous feeding begins.

4.3 Characteristic of the egg envelope, eye pigmentation, melanophore pattern and fins development in *Notopterus notopterus* and *Osteoglossum bicirrhosum* with comparison to other osteoglossomorphs

The egg of *Notopterus notopterus* has many external ridges which are centred around the single micropyle located at the animal pole. Mookerjee and Marzumdar (1946) also stated that eggs of *N. notopterus* show a “groove like line, radiating from the micropyle”. Based on the four classifications of micropyle described by Kunz (2004, modified from Riehl, 1991), the micropyle of *N. notopterus* belongs to type number 1: the eggs surface displays a spiralling pattern of ridges partially ending in the micropylar region (Riehl and Kokoschka, 1993). The pattern of the furrows on the surface of these species is radial, running from the animal to the vegetal pole (Kunz, 2004). The egg of the catfish *Sturisoma aureum* of the family Loricariidae (Riehl and Patzner, 1991) and the cyprinid *Barbus conchoni* of the family Cyprinidae (Amanze and Iyengar, 1990) also possess a micropyle with grooves and ridges directed toward the micropylar canal. The chorion in two other osteoglossomorphs *Pollimyrus isidori* (Diedhiou et al., 2007b) and *Pantodon buchholzi* (Britz, 2004) appears smooth at binocular magnification.

Eye pigmentation in both *N. notopterus* and *O. bicirrhosum* commence within the egg envelope once five or six days old. It is proven by Argumedo (2005) that at stage I, 10.5 mm long embryos *O. bicirrhosum* show first signs of pigmented eyes inside of the egg envelope. In contrast to *Pollimyrus isidori* (Diedhiou et al., 2007b), first indicators of pigmented eyes emerged on the fourth day after hatching in *P. adspersus* (Diedhiou et al., 2007a) and *Petrocephalus soudanensis* (Kirschbaum, 2006) and a few hours after hatching in fishes of the *Paramormyrops magnostipes*-complex (Nguyen, 2011).

The onset of melanophore formation in *N. notopterus* emerges around the forehead prior to hatching, at the age of five days. In contrast to this, a massive black pigmentation first appears to be covering the brain region of *O. bicirrhosum* on the first day after hatching. This was also found by Argumedo (2005): black melanophores were first seen after hatching, in stage II fry in 17–20 mm long individuals.

In mormyrids, the first melanophores appear in *Pollimyrus isidori* the day after hatching in the dorsal head epidermis (Diedhiou et al., 2007). In fishes of the *Paramormyrops magnostipes*-complex, black melanophores developed one day after hatching on the top of the head (Nguyen, 2011). In *Pantodon buchholzi*, black melanophores were observed for the first time on the head areas, on the body and on a reticulate pattern on the dorsal region of the yolk-sac (Britz, 2004). The free embryos of *Hiodon alosoides* lack melanophores completely, although occasionally melanophores could appear on the mid-dorsal surface (Battles and Sprules, 1960). As for

Hiodon tergisus, Synder and Douglas (1978) reported that some branched melanophores appeared a few days after hatching, distributed on the ventral half of the yolk sac.

Some colouring is present in *N. notopterus*, especially starting at the age of 52 days until 92 days. In contrast, *Pantodon buchholzi* shows a unique coloration in the larval period only (Britz, 2004).

The development of pectoral, dorsal, anal, caudal, and fins already starts prior to the onset of exogenous feeding in *Notopterus notopterus*. This is similar to *Pollimyrus isidori*, *P. adspersus*, *Mormyrus rume probocirostris* (Diedhiou et al., 2007b) and also *Pantodon buchholzi* (Britz, 2004). In three other mormyrids *Campylomormyrus tamandua*, *Petrocephalus soudanensis* and *Hippopotamyrus pictus*: the demarcation of the dorsal and anal fins emerges later, during the larval period (Diedhiou et al., 2007b). Similar to *N. notopterus* and other forementioned mormyrid species, the development of fins in *Osteoglossum bicirrhosum*, starts with pectoral fins, followed by dorsal, anal, caudal and at last with the pelvic fins.

4.4 Comparison of periods and phases in development of *Notopterus notopterus* and *Osteoglossum bicirrhosum*

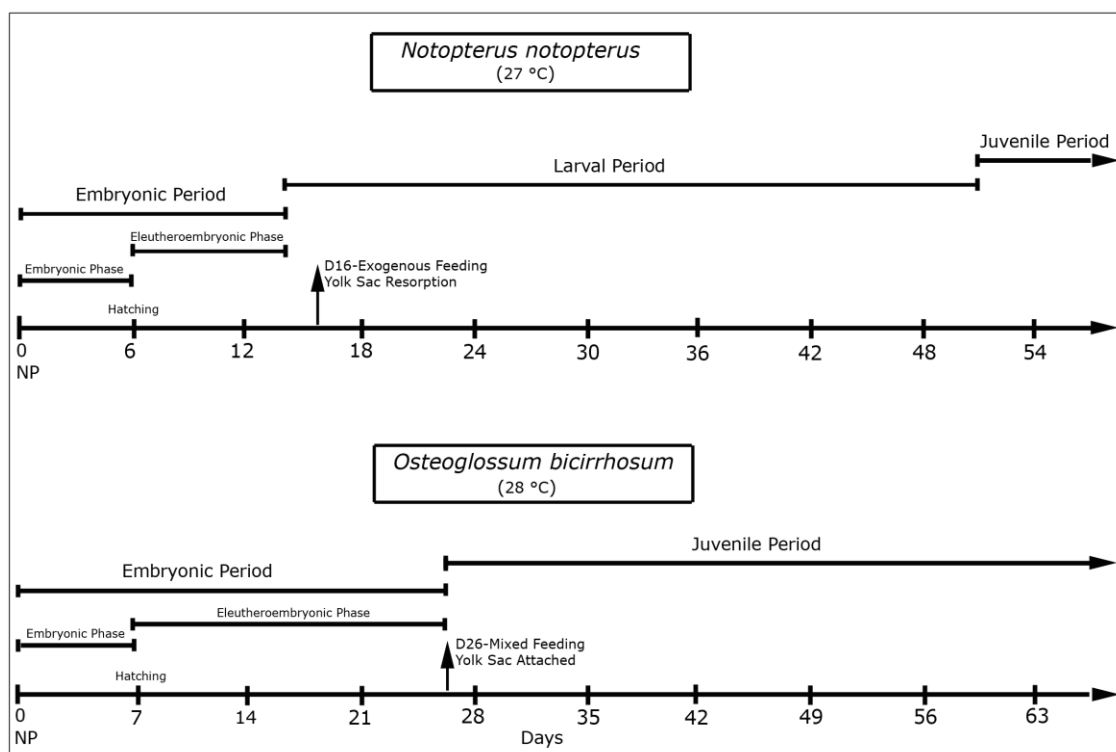


Fig. 65. Comparison of periods and phases in the development of *Notopterus notopterus* and *Osteoglossum bicirrhosum* (based on terminology of Balon, 1975). Neural plate (NP) as the beginning of the time scale.

In this study, a nearly complete series of development stage in *Notopterus notopterus* and *Osteoglossum bicirrhosum* is described for the first time. Figure 65 illustrates the comparative development as divides into periods and phases in both *N. notopterus* and *O. bicirrhosum*.

Neurulation, the externally well visible development of the neural plate is used as the standard onset of the time scale in both development courses for comparative purpose. The neural plate (NP) stage in *N. notopterus* sets in at 29 hours after spawning and is observed in *O. bicirrhosum* at 8 hours after collecting time.

4.4.1 Periods

There are three periods described in **Figure 65**: the embryonic period, the larval period and the juvenile period. In *N. notopterus* compared to *O. bicirrhosum*, the embryonic period lasts shorter and the onset of juvenile period starts much delayed. The larval period lasts long before shifting to the juvenile period in *N. notopterus*. The larval period in *N. notopterus* is characterized by obliteration of the embryonic fin fold, a fully resorbed yolk sac, a distinct larval coloration and the onset of exogenous feeding. The temporary embryonic fin fold appears after 95 hours and lasts until day 13 in *N. notopterus*. The larval period is then followed by the juvenile period starting on day 51 and lasting until the age of 18 months.

The larval period is absent in *O. bicirrhosum* since none of the temporary organs were found. This is in contrast to Argumedo (2005) who mentioned the existence of the larval period in *O. bicirrhosum*, probably by implicitly using another definition of a larval phase.

The start of the juvenile period is characterized by the the onset of mixed feeding in *O. bicirrhosum*, which was observed at day 26. At the age of 36 days with a total length of 45 mm, the juveniles of *O. bicirrhosum* have already accomplished the formation of the definite organs such as well-developed pelvic fins, barbels, and the onset of scale formation, including the almost entirely absorbed yolk sac and the completed juvenile colouring. At this point the juveniles are already capable of leaving the parent's mouth and try to swim individually in their natural habitat. Around day 100, *O. bicirrhosum* reaches a length of 125 mm with the fully absorbed yolk-sac and the juveniles resemble young adults. The juvenile period in *O. bicirrhosum* lasts for a very long time until it turns into an adult. *O. bicirrhosum* is fully sexually mature from the age of 28 months on.

The absence of the larval period in *O. bicirrhosum* leads to a conclusion that this species undergoes a direct development, while *N. notopterus* undergoes an indirect development including the larval period.

4.4.2 Phases

The embryonic period consists of two phases: the embryonic phase and the eleutheroembryonic phase. The embryonic phase indicates the development within the egg envelope until hatching occurs. This phase lasts nearly similarly long in both species.

The eleutheroembryonic phase in *N. notopterus* commences shortly after hatching and lasts until the onset of exogenous feeding. While, the eleutheroembryonic phase in *O. bicirrhosum* starts after hatching and lasts until the first mixed feeding while the yolk sac is still attached. The eleutheroembryonic phase in *O. bicirrhosum* is almost twice as long as in *N. notopterus*.

4.4.3 General comments

A large yolk sac in *O. bicirrhosum*, allows it to protect its offspring during and after mouth breeding, especially providing massive food supply endogenously. Producing large yolky eggs, the embryos of *O. bicirrhosum* tend to stay longer inside the egg envelope which enables further protection by parental care.

Possessing a large yolk sac allows the embryos to instantly structure the whole suite of permanent organs without being required to modify any temporal larval structures (Balon, 1984b). Oppenheimer (1970) stated that, the greater the amount of yolk in the eggs, the less likely parental care will be continued after the fry are released.

Mixed feeding in *O. bicirrhosum*, referring to exogenous feeding alongside with the yolk-sac still attached. This is in accordance with Balon (1984b) who mentioned that often the initial action of a juvenile period in direct developing organisms is categorized by mixed feeding of varying duration. Mixed feeding is delayed for around 10 days in *O. bicirrhosum* compared to *N. notopterus*. Elimination of the vulnerable larva and metamorphosis may facilitate direct development into a juvenile that is relatively advanced at the first oral feeding (Balon, 1999). Mixed feeding also occurs in the mouth breeding *Cyphotilapia frontosa* (Cichlidae) starting with free embryos until early juvenile (Balon, 1999). In a cichlid species *Labeotropheus*, the eleutheroembryo develops directly into a juvenile without metamorphic stages, forming advanced structures like fins, skeleton and pigments at a time when a large yolk sac is still present

(Balon, 1977). As found in most species, the juveniles will soon live in shallow nursery habitats, which are unreachable to larger piscine predators (Balon, 1999).

Different models of life history for fish were proposed by some authors. Winemuller (2005) illustrated a triangular model: 1) opportunistic – short generation times and small body size, producing a large number of eggs with no parental care (comparable to r-strategist), 2) periodic species – long-living individuals producing lots of eggs, but provide no parental care, and 3) equilibrium species – living fairly long with few offspring and provide parental care (comparable to K-strategist). Based on his concept consequently *N. notopterus* belongs to the opportunistic model and equilibrium model matches *O. bicirrhosum*.

Egg size in *Notopterus* is also shown to be not that diminutive as in typical textbook examples of r-strategic reproduction, since this species produces medium-sized eggs with parental care. *N. notopterus* therefore is classified as an intermediate species with respect to interpretation of a reproductive strategy, while *O. bicirrhosum* is classified as a K-strategic. Rather, each species apparently evolves a unique suite of adaptive characters, in order to survive in their environment and to protect their offspring. This is well demonstrated in the wide range of reproductive styles among Osteoglossomorpha – and the terminology and stage definitions of early ontogenetic development in fish established by Balon (1975a, b) is well suited to characterize these various reproductive styles in the light of adaptiveness.

5 CONCLUSION

This study essentially contributes to the knowledge of the reproduction and the ontogenetic development in two species of Osteoglossomorpha belonging to two families: *Notopterus notopterus* (Notopteridae) and *Osteoglossum bicirrhosum* (Osteoglossidae).

The only comparable in-depth study is available in Mormyridae (*Pollimyrus isidori*).

For the first time the ontogenetic development is described in detail and fairly complete in the mouth breeder *Osteoglossum bicirrhosum*. This ontogeny is characterized by the absence of a larval period and thus representing direct development, whereas *Notopterus notopterus* represents a more typical indirect development.

A broader comparison of the various reproductive styles in Osteoglossomorpha, also based on ontogenetic data of previous authors, characterizes *Osteoglossum bicirrhosum* as “k-strategic” and *Notopterus notopterus* as “intermediate” with respect to interpretation of a reproductive strategy.

Thus this study has considerably increased our understanding of reproduction and ontogenetic development in the basal and very diverse taxon Osteoglossomorpha

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Hiermit erkläre ich, die Dissertation selbständig und nur unter Verwendung der angegebenen Hilfen und Hilfsmittel angefertigt zu haben.

Ich versichere, dass die Dissertation bisher weder in Teilen noch als Ganzes einem Promotionsverfahren zugrunde lag.

Ich habe mich nicht anderwärts als Doktorand beworben und besitze keinen entsprechenden Doktorgrad.

Ich erkläre die Kenntnisnahme der dem Verfahren zugrunde liegenden Promotionsordnung der Landwirtschaftlich-Gärtnerischen Fakultät der Humboldt-Universität zu Berlin.

Berlin, den 7. Januar 2013

Honesty Yanwirsal